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HALF-LIVES OF DIFFERENT SIZED mRNAs FOR THE PKA SUBUNIT RIα ARE REGULATED DIFFERENTLY IN RESPONSE TO INHIBITION OF TRANSCRIPTION AND TRANSLATION

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The RIα mRNA level is induced 3-5 times by FSH or cAMP analogs in primary cultures of rat Sertoli cells. In rat tissues, the RIα gene gives rise to three different mRNAs of different size: 3.2, 2.9 and 1.7 kb. In the present study we report that the 1.7 kb transcript has a shorter half-life than the two other mRNAs. In cells which had been pre-stimulated with a cAMP analog, inhibition of transcription stabilizes the two larger, but not the smaller sized RIα mRNA. However, in contrast, inhibition of protein synthesis stabilizes all the RIα mRNAs. Thus, degradation of various mRNAs coding for the same protein reveals different dependencies on transcription and translation.

The regulatory subunit type Iα of cAMP-dependent protein kinases (PKA) is expressed in all cell-types which have been examined so far, and both the protein and the nucleic acid sequences in different species are highly conserved (1-6). The RIα gene gives rise to two or three mRNAs of different size depending on species. In human tissues, the two mRNAs present (3.0 and 1.5 kb) have identical protein-coding regions, but interestingly, the longer mRNA contains a 1.5 kb 3'non-translated (3'nt) region which is not present in the shorter transcript. These mRNAs are formed by utilization of alternative poly adenylation site signals (7). The distribution of large and small RIα mRNAs varies between cell-types within the same species, indicating that the different mRNA forms are subject to differential regulation (8-11). The function of different 3'nt regions in mRNAs coding for identical proteins is unknown, but they might control the translation efficiency, or be involved in differential regulation of mRNA stability.

In primary cultures of rat Sertoli cells the RIα, RIIα, RIIß and Cα subunits of PKA are present (8). We have previously reported that the three various subunits of PKA are differentially regulated by FSH or cAMP (12,13).

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The purpose of the present study was to examine whether the different sized RIα mRNAs revealed different stabilities, and to what extent inhibitors of RNA and protein synthesis influence RIα mRNA degradation. We show that the smaller (1.7 kb) mRNA is degraded more rapidly than the larger (2.9 and 3.2 kb) RIα mRNA forms, and that their stabilities are influenced differently by inhibitors of RNA and protein synthesis.

MATERIALS AND METHODS

Primary cultures of rat Sertoli cells were prepared as previously described (14). Experiments were started on day 4 after plating. Cells were stimulated with dibutyryl-cAMP (dbcAMP, Sigma D-0627) for 12 h. To remove the cAMP analog after treatment, cultures were washed three times with 5 ml MEM which was supplemented with the various inhibitors when appropriate. The cells were then supplied with fresh medium, and incubation continued with or without actinomycin D (5μg/ml; Sigma A-4264), cycloheximide (5μg/ml; Sigma C-6255) or 5,6-dichlorobenzimidazole riboside (DRB, 150μM; Sigma D-5893). The medium was changed 12 h prior to start of the experiments.

Total RNA was extracted by homogenization in guanidium isothiocyanate (GTC) followed by centrifugation through a CsCl gradient and phenol/chloroform extraction (14).

Northern analysis was performed as described before, using 20 μg total RNA per. lane (14). The RIα probe was a 0.8 kb human EcoRI fragment (5).

RESULTS

Results in fig. 1 show that the 1.7 kb RIα mRNA was degraded faster than the 3.2 and 2.9 kb mRNAs when transcription was inhibited by either actinomycin D or DRB. The half-life of the 1.7 kb mRNA was approximately 5 h, whereas that of the two larger mRNAs was much longer (> 24 h). The decay of the various sized RIα mRNAs was similar whether actinomycin D or DRB were used to inhibit RNA synthesis.

In fig. 2 we examine the half-lives of the three RIα mRNAs in cells which had been treated with dbcAMP for 12 h to induce the messages. Removal of dbcAMP was associated with a rapid decrease of all the RIα mRNAs (half-lives approximately 6 h). Whereas actinomycin D clearly stabilized the larger RIα mRNAs (fig. 2, upper left panel), the decay of the smaller RIα mRNA was accelerated (fig 2, upper right panel). The Northern data shown both in figs. 1 and 2 also indicate that the smaller RIα mRNA actually consists of two bands, and that only the larger of these two mRNAs is degraded in the presence of actinomycin D or DRB.

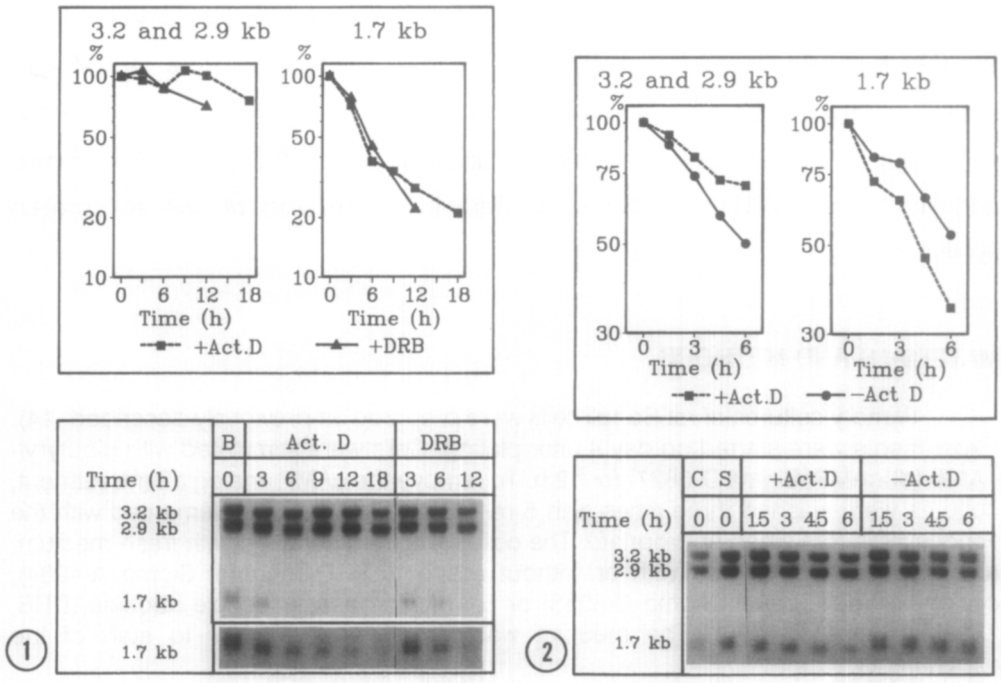

Figure 1. The smaller (1.7 kb) RIα mRNA is degraded faster than the two larger (2.9 and 3.2 kb) RIα mRNAs.
Sertoli cell cultures were incubated in the presence of actinomycin D (Act.D, 5μg/ml) or DRB (150μM). Total RNA was extracted at various time points thereafter and analyzed by Northern technique. The upper panel illustrates results obtained by densitometric scanning of the autoradiograms in the lower panel. The basal level (B) before addition of RNA synthesis inhibitors was defined as 100%. The 1.7 kb RIα message is shown at two different exposures of the filter to visualize the relative low levels without overexposing the 2.9 and 3.2 kb messages.

Figure 2. Actinomycin D stabilizes the two larger (3.2 and 2.9 kb) but not the smaller (1.7 kb) RIα mRNA after stimulation with dbcAMP.
Sertoli cell cultures were incubated for 12 h in the absence (C) or presence (S) of dbcAMP (10^{-4}M). Then the cultures were washed as described in materials and methods, and incubation was continued for 6 h in the absence of cAMP analog and in the presence and absence of actinomycin D (Act.D, 5μg/ml). Total RNA was extracted at various times and subjected to Northern analysis. The upper panel depicts values obtained by densitometric scanning of the autoradiogram shown in the lower panel. The results are shown relative to the stimulated levels, which are defined as 100%.

The results presented in fig. 3 show that rapid degradation of all the RIα mRNAs is dependent on protein synthesis. The cultures were first treated with dbcAMP for 12 h to induce the RIα mRNAs. Removal of cAMP was associated with a rapid decrease of all RIα mRNAs towards basal. Incubation with the protein synthesis inhibitor cycloheximide completely blocked the decrease of the larger RIα mRNAs and actually further increased the smaller (1.7 kb) RIα mRNA above the level seen after cAMP treatment (fig. 3). Combined treatment with both cycloheximide and actinomycin D prevented the degradation of all RIα mRNAs.

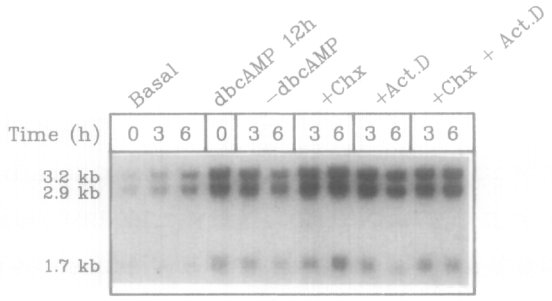

Figure 3. Degradation of the RIα mRNAs is dependent on protein synthesis.
Sertoli cells were stimulated for 12 h with dbcAMP (10^{-4}M). Then the cultures were washed and incubation was continued in the absence of cAMP analog and in the presence of actinomycin D (Act.D, 5μg/ml) or cycloheximide (Chx, 5μg/ml), or a combination of these compounds. Total RNA was isolated 3 and 6 h after washing and subjected to Northern analysis.

DISCUSSION

In the present study we show that the half-life of the 1.7 kb RIα mRNA is shorter than that of the 2.9 and 3.2 kb mRNAs in primary cultures of rat Sertoli cells. We further demonstrate that after dbcAMP stimulation, the larger RIα mRNAs are stabilized by inhibition of transcription whereas the smaller RIα mRNA is not. Our data indicate that the 3'nt parts of the 2.9 and 3.2 kb RIα mRNAs are involved in the distinct stabilization of these transcripts by inhibitors of transcription.

The half-lives of the RIα mRNAs was not affected by pre-stimulation with dbcAMP, and inhibition of transcription with actinomycin D or DRB gave virtually identical results. These inhibitors supposedly act through different mechanisms. Whereas actinomycin D blocks transcription by intercalating in the DNA helix (15), DRB causes premature termination or inhibits initiation of transcription (16-18). Our results are in contrast to the recent report of Lange-Carter and Malkinson (19). They found that the smaller RIα mRNA was more stable than the larger RIα mRNAs in two mouse lung epithelial cell lines. This indicates cell and/or species specific differences in the regulation of degradation of specific mRNAs.

So far, only the cDNA corresponding to the short rat RIα mRNA has been cloned (4). However, the long transcript (3.0 kb) of human RIα contains several "AUUUA" sequences which are present in several short-lived mRNAs, including c-fos and c-myc mRNAs (20). Such "AUUUA" motifs are not present in the 3'nt part of the 1.5 kb human RIα mRNA (7). Since repeats of the "AUUUA" motif have been shown to destabilize several mRNAs, one could expect shorter half-lives of the two longer RIα

mRNAs than of the shorter one. This is certainly not the case in rat Sertoli cells, but consistent with the findings in murine lung epithelial cell lines (19). However, it has been reported that the 3'nt part of c-fos mRNA was not destabilizing in the presence of RNA synthesis inhibitors (21), whereas such inhibitors did not affect the destabilizing effect of the 3'nt part of c-myc mRNA (22). This indicates that several factors interact to stabilize or destabilize mRNAs containing "AUUUA" sequences in their 3'nt parts.

In rat Sertoli cells, increase of RIα mRNA levels by cycloheximide has been demonstrated (13). In the present study we show that degradation of the RIα mRNAs, particulary that of the 1.7 kb mRNA, was blocked by cycloheximide, thus indicating that increased levels of RIα mRNA with cycloheximide at least partly is caused by an effect on mRNA stability. The increase in RIα mRNA was also observed with anisomycin or puromycin (data not shown), two inhibitors of translation which act through other mechanisms than cycloheximide (23). Stabilization of specific mRNAs by protein synthesis inhibitors has been reported in several systems (24,25). Decreased mRNA degradation due to disappearance of a regulatory protein with high turnover is one of the theories for the mechanism behind this effect. This theory is supported by the recent paper from Koeller et al (26), who showed that the induction of c-fos and transferrin receptor mRNAs after inhibition of translation was due to disappearance of a trans-acting factor, and not the inhibition of translation of these transcripts per se. Degradation of all the RIα mRNAs could be mediated by a similar protein, since inhibition of protein synthesis stabilized all the RIα mRNAs. However, degradation of the larger RIα mRNAs must be dependent on other factors as well, since inhibition of RNA synthesis stabilized the two larger, but not the smaller RIα mRNAs. The additional factor could be a labile protein encoded by a short-lived mRNA, or a short-lived RNA molecule itself. We have previously shown that inhibitors of transcription stabilize mRNAs for the PKA subunits RIIα, Cα and RIIβ in rat Sertoli cells (27), and that a short-lived RNA molecule probably stabilizes nuclear RIIβ mRNA by a mechanism not involving translation (28).

The biological significance of mRNAs with identical protein coding regions, but with different non-translated parts is not known. The fact that the half-lives of the RIα mRNAs are subject to differential regulation both in murine lung epithelial cell lines as well as in rat Sertoli cells implies that they may have distinct functions. Whether this involves sub-cellular localization of mRNAs, regulation of translation-efficiency or some other unknown functions remains to be shown.

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