Stabilization of the mRNA for the uncoupling protein thermogenin by transcriptional/translational blockade and by noradrenaline in brown adipocytes differentiated in culture: a degradation factor induced by cessation of stimulation?

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The stability of the mRNA coding for the uncoupling protein thermogenin was investigated in mouse brown-fat cells differentiated in culture. After 7 days in culture, the cells were stimulated for 24 h with noradrenaline, and a high level of thermogenin mRNA was then observed. If noradrenaline treatment was continued, the mRNA level remained high, but, upon withdrawal of noradrenaline, the level decreased rapidly, with a half-life of only 2.7 h. The presence of transcriptional (actinomycin) or translational (cycloheximide) inhibitors prolonged the apparent half-life by about 50%. The presence of noradrenaline during transcriptional blockade led to a further stabilization of thermogenin mRNA. It was concluded that an induced (or short-lived) gene product is important for thermogenin mRNA degradation. Direct interaction of noradrenaline with the cultured brown adipocytes could apparently not mimic the paradoxical destabilization of thermogenin mRNA *in vivo*, previously observed in the cold-exposed mouse [Jacobsson, Cannon and Nedergaard (1987) FEBS Lett. **224**, 353–356], indicating significant differences between the systems *in vitro* and *in vivo*.

INTRODUCTION

It is generally considered that the cellular level of mRNA coding for a given protein is a (or the) major determinant for the rate of synthesis of that protein. The level of mRNA is the resultant of transcriptional activity and degradation rate. Whereas considerable experimental effort has been channelled into an understanding of the regulation of transcription, comparatively little effort has been made concerning the understanding of the regulation of mRNA stability and degradation in eukaryotes. It is, however, clear that mRNA decay is not a random and unselective process, but rather a highly specific and regulated process [1–9].

One general conclusion concerning regulation of mRNA stability is that conditions (e.g. hormonal stimulation) which lead to an increased expression of a gene also lead to stabilization of the relevant mRNA species [1,6]. In brown adipose tissue, the gene for the uncoupling protein thermogenin [10,11] is highly induced during cold acclimation [12–24]. It was therefore unexpected to observe that the same physiological condition (cold) that is associated with this high induction of thermogenin gene expression was also associated with a dramatic destabilization of thermogenin mRNA: the half-life (after transcriptional inhibition) was about 18 h in mice returned to warm conditions, but as short as 3.2 h in mice remaining in the cold [19]. Such a short thermogenin mRNA half-life (3.7 h) in mice in the cold was also observed by Tvrdik et al. [25] (but longer half-lives have been observed in rats [23]).

The cellular mechanism behind this cold-induced destabilization of thermogenin mRNA has so far not been elucidated. However, the development of experimental conditions allowing for differentiation of brown-fat cells in culture and for induction of thermogenin gene expression in this system [26] has now enabled us to address this question *in vitro*.

Cold exposure of animals is associated with an increased sympathetic stimulation of the tissue [27], leading to enhanced noradrenaline release in the tissue [28]; in experiments *in vitro*, noradrenaline has been shown to be competent in stimulation not only of thermogenesis [29] but also of cell division [30] and thermogenin gene expression [26]. It is therefore a reasonable suggestion that the destabilization of thermogenin mRNA during cold exposure would also be due to noradrenaline stimulation of the cells. We have here utilized the brown-fat cell-culture system in order to examine the ability of noradrenaline to destabilize thermogenin mRNA.

We found that noradrenaline did not destabilize thermogenin mRNA in the system *in vitro*. Rather, we saw a stabilization due to noradrenaline treatment, indicating significant differences between the systems *in vivo* and *in vitro*. Furthermore, we observed that transcriptional and translational inhibition also led to a stabilization of thermogenin mRNA, indicating the presence of inducible (or short-lived) factors (nucleases), responsible for the observed rapid thermogenin mRNA degradation.

MATERIALS AND METHODS

Cell isolation and culture

Brown-adipose-tissue precursor cells were isolated as previously described [31] from cervical, axillary and interscapular brown adipose tissue of 4-week-old male NMRI mice, obtained from a local supplier (Eklunds). A 0.2 ml portion (equivalent to two-fifths of the total number of precursor cells obtained from one mouse) of the pooled final cell suspension was inoculated into

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each well (35 mm diameter), which contained 1.8 ml of culture medium consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn-calf serum, 4 nM insulin, 10 mM Hepes, 50 i.u./ml penicillin, 50 μg/ml streptomycin and 25 μg/ml sodium ascorbate [26,32]. The cells were grown at 37 °C in an atmosphere of 8% CO_2 in air. On day 1 after inoculation, the culture medium was removed and the cells were rinsed with 2 ml of DMEM before addition of 2 ml of fresh culture medium. On day 3, the medium was exchanged with fresh medium without rinsing. On day 7 (when the cell cultures were confluent), the cells were exposed to noradrenaline [(−)-Arterenol (bitartrate salt), Sigma] for 24 h (1 μM, added at 0 and 8 h). After this pretreatment period, the medium was discarded, and the cells were washed with DMEM. New medium was added, and the cells were treated as described in the legends to the Figures. Additions included actinomycin D (containing mannitol; water-soluble, freeze-dried; Sigma) and cycloheximide (Sigma).

RNA isolation and analysis

For RNA extraction, the cells were washed once with NaCl and then dissolved in 0.8 ml of hot guanidine hydrochloride extraction buffer [15]. Total RNA was extracted [17]. The RNA concentration was determined in a spectrophotometer at 260 nm; the 260/280 ratios were routinely higher than 1.8. Total RNA yields after pretreatment (day 8) were 21 ± 3 μg RNA per 35 mm well (mean of 10 different series). Samples (5 μg) of total RNA, denatured with formamide/formaldehyde, were electrophoresed in a 1.25%-agarose gel containing 20 mM Mops, 6.7% formaldehyde and 0.1 mg/ml ethidium bromide, in a 20 mM Mops buffer containing 50 mM sodium acetate and 10 mm EDTA. RNA was blotted on to Hybond nylon membranes in 20 × SSC (saline sodium citrate buffer: 0.3 M trisodium citrate, 3 M NaCl, pH 7.0), according to the Northern-blot procedure [15]. A positive control for thermogenin mRNA (brown-fat RNA isolated from mice exposed to 4 °C for 24 h) and a negative control (brain RNA isolated from the same mice) were included in all the Northern blots. The blots were hybridized with a [^{32}P]dCTP-random-primed cDNA clone corresponding to mouse thermogenin [15], as previously described [33]. For quantitative analysis, the bands were scanned with a Molecular Dynamics Computing Densitometer 300 S or PhosphorImager 425 S, and the results were expressed as percentages of the level of thermogenin mRNA obtained at the end of the 24 h noradrenaline pretreatment period. The blots were not rehybridized with a cDNA probe for a 'constitutive' mRNA (such as β-actin); as also the level of such mRNAs would be influenced by the agents used here [34], it would not be possible to compensate for unequal RNA application by this method. Rather, equal RNA application was checked by observation of ethidium bromide-stained blots under u.v. light (cf. Figure 4), and all experimental data shown are means from the indicated numbers of independent experiments.

RESULTS

In order to study thermogenin mRNA stability, we have used mouse brown adipocytes differentiated in culture. For the present experiments, brown-adipocyte precursors were grown for 7 days in culture, during which time they differentiated into brown adipocytes. These cells express the thermogenin gene at very low levels in the unstimulated state, but the level of thermogenin mRNA (and of the protein itself) can be dramatically increased by adrenergic stimulation [26,35]. In order to obtain the substantially elevated thermogenin mRNA level required for the present experiments, the differentiated cells were pretreated with noradrenaline for 24 h. This induced level of thermogenin mRNA was thus the starting point for analysis of thermogenin mRNA stability.

Half-life of thermogenin mRNA following cessation of stimulation

We initially estimated the half-life of thermogenin mRNA *in vitro* after cessation of the adrenergic stimulation. For this purpose, we either re-added noradrenaline to the cell cultures after the 24 h noradrenaline pretreatment period, or the noradrenaline treatment was terminated at this time point. The thermogenin mRNA levels were then measured at different times thereafter in order to determine the rate of degradation of the mRNA.

As seen in Figure 1, in the cells to which noradrenaline was re-added, thermogenin mRNA levels remained high (Figure 1; ●) and practically stable over the analytical period [the nominal half-life was 26 h, but, as the correlation coefficient was very low (0.32), the level must be considered to be stable]. This indicated that a steady-state level of thermogenin mRNA had already been reached during the 24 h of noradrenaline pretreatment. In contrast, in the cell cultures which were no longer adrenergically stimulated, thermogenin mRNA levels rapidly declined (Figure 1; ○). It would, however, seem that a lag time of some 2 h could be discerned, and thereafter a tendency to an accelerated degradation with time was observed; this tendency was observed in 4 independent experiments (results not shown). When all data points were used for analysis of half-life, a value of 2.2 h was obtained in the experiment shown [the most rapid degradation phase (the last 6 h) corresponded to a half-life of 1.5 h]. As a

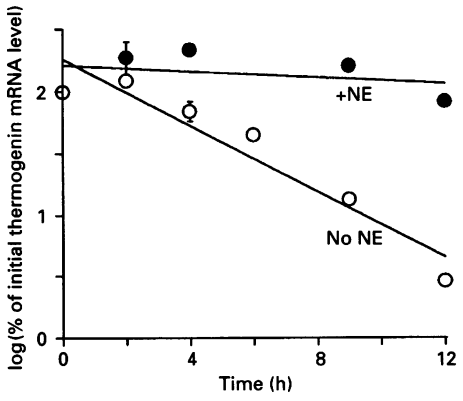

Figure 1 Effect of noradrenaline (NE) removal on thermogenin mRNA levels in cultured brown adipocytes

Cultured cells were grown for 7 days and then pretreated for 24 h with noradrenaline (1 μM). After this 24 h period, the culture medium was discarded, and the cells were washed and fed either with fresh culture medium alone (○) or with fresh culture medium and a re-addition of noradrenaline (●). The cells were harvested for thermogenin mRNA analysis at the times shown, as described in the Materials and methods section. The mean initial thermogenin mRNA level obtained at the end of the 24 h noradrenaline pretreatment period was set to 100%, and the other values are expressed relative to this (note the logarithmic y axis). The results shown here are from one experiment. Symbols are mean values obtained from duplicate wells, and error bars indicate individual values, except in cases where the variation was smaller than the symbol size. Lines were drawn according to least-squares analysis, and the half-lives were calculated from the slopes. In the presence of noradrenaline, the apparent half-life was 26 h ($r = 0.32$); in the absence of noradrenaline, the half-life was 2.2 h ($r = 0.95$).

mean of 4 independent experiments, a half-life of 2.7 h was calculated (all time points included) (Table 1).

This remarkably short half-life upon cessation of stimulation may either indicate a continuous rapid turnover of thermogenin mRNA during adrenergic stimulation, or it may imply that, upon termination of stimulation, a rapid degradation process is induced; the data from this experiment alone do not allow for distinction between these possibilities.

Effect of transcriptional blockade on thermogenin mRNA stability

In order to investigate if the rate of thermogenin mRNA degradation in the absence of noradrenaline was identical with that obtained after complete cessation of gene transcription, we used the transcription inhibitor actinomycin D. Previous results had demonstrated that actinomycin is able to block transcription of the thermogenin gene both *in vivo* [17] and *in vitro* [26]. In order to ensure that the lowest possible dose of actinomycin which would effectively block transcription of the gene was used, we exposed differentiated brown adipocytes to actinomycin at the concentrations indicated in Figure 2 and then stimulated with noradrenaline. The results showed that actinomycin prevented the normal noradrenaline-induced increase in thermogenin mRNA levels in a dose-dependent manner, and, at an actinomycin concentration of 1000 ng/ml, noradrenaline-induced gene transcription was fully blocked. Consequently, actinomycin was used at this lowest fully effective concentration in the following experiments; this concentration of actinomycin had no visible effect on cell appearance, and the yield of RNA after 10 h with this actinomycin concentration was not statistically different from the initial yield (cf. legend to Table 2).

Thus, after the 24 h pretreatment period with noradrenaline, we either treated the cells with actinomycin or did not treat them, and thermogenin mRNA levels were then determined. In cells which had not received actinomycin, thermogenin mRNA levels again declined rapidly (Figure 3, ○), with a half-life of 3.7 h in the experiment shown. In contrast, thermogenin mRNA levels in the cells which had received actinomycin declined more slowly (Figure 3, □), with a half-life of 7.9 h in the experiment shown. The mean half-life value based on 3 experiments was 7.4 h (Table 1). Thus, thermogenin mRNA was degraded more slowly in the presence of actinomycin than in the mere absence of adrenergic stimulation. This fact implies that short-lived or induced transcription-dependent factors are responsible for the rapid breakdown of thermogenin mRNA upon cessation of stimulation. Closer scrutiny of Figure 3 would seem to indicate that it was especially the accelerating phase of the degradation that was affected by actinomycin; a similar observation was made in all 3 independent experiments (results not shown). This could be interpreted to indicate that cessation of stimulation leads to induction of a degrading factor.

It should also be noted (Figure 3) that transcriptional blockade did not lead to a complete inhibition of thermogenin mRNA breakdown; thus other, more long-lived, factors must be responsible for the remaining degradation of thermogenin mRNA. These factors must be very long-lived, as no tendency to a decrease in the rate of degradation could be observed during the 9 h of experimentation.

Effect of translational blockade on thermogenin mRNA stability

In order to investigate if the stabilizing effect of actinomycin on thermogenin mRNA could be due to an inhibition of production

Table 1 Thermogenin mRNA half-lives

Cultured cells were grown and treated according to the legends in Figures 1, 3 and 5. Values are means ± S.E.M. of 3 or 4 independent experiments performed in duplicate wells. ** and *** indicate statistically significant effects of the different treatments versus no treatment (— NE) ($P < 0.01$ and $P < 0.001$, respectively; Student's t test); † indicates a significant effect of noradrenaline on the half-life in the presence of actinomycin alone ($P < 0.05$). Due to the low value of the correlation coefficient for cell cultures treated continuously with noradrenaline (+ NE), the estimation of a half-life for thermogenin mRNA in this condition is not statistically meaningful, and the nominal value obtained is therefore given in parentheses.

Treatment	Half-life	r	n
+ NE	(19.7 ± 5.3)**	0.56 ± 0.13	3
— NE	2.7 ± 0.4	0.96 ± 0.10	4
+ Act	7.4 ± 0.6***	0.94 ± 0.04	3
+ Act + NE	10.1 ± 0.8***†	0.92 ± 0.04	3

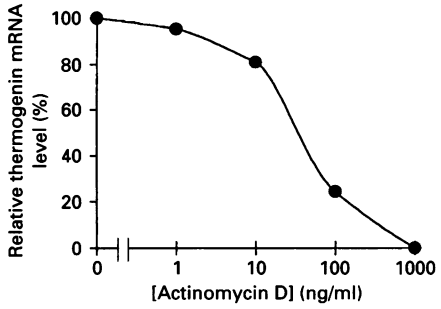

Figure 2 Effect of actinomycin on noradrenaline-induced thermogenin gene expression in cultured brown adipocytes

Cells were grown for 7 days and then exposed to actinomycin at the concentrations shown. Noradrenaline (1 μM) was added to the cells 10 min later. Cells were harvested 4 h after the addition of noradrenaline. The mean thermogenin mRNA level obtained after the 4 h noradrenaline treatment period in the absence of actinomycin was set to 100%, and the other values are expressed relative to this. Symbols are mean values obtained from duplicate wells; the variation was smaller than the symbol size. No thermogenin mRNA was seen in the absence of noradrenaline.

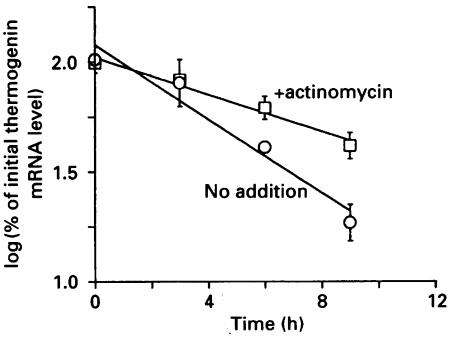

Figure 3 Effect of actinomycin on thermogenin mRNA stability in cultured brown adipocytes

After the 24 h noradrenaline stimulatory period, the culture medium was discarded and replaced either with fresh culture medium alone (○) or with fresh culture medium plus actinomycin (final concn. 1 μg/ml) (□). Analysis and representation of results are as described in Figure 1. After noradrenaline removal, the half-life was 3.7 h ($r = 0.94$); after noradrenaline removal with the addition of actinomycin, the half-life was 7.9 h ($r = 0.94$).

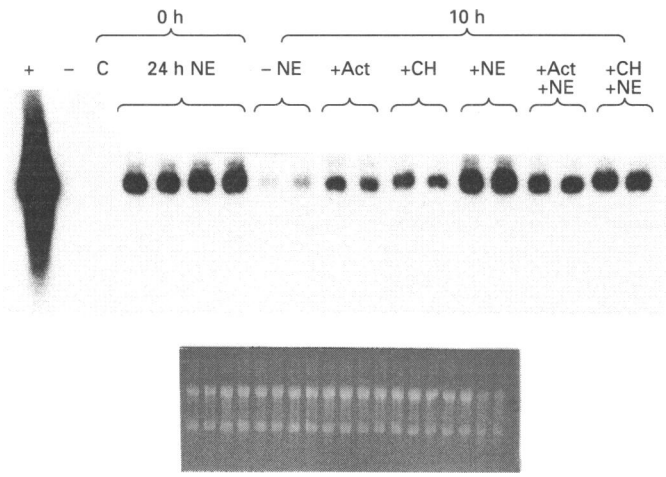

Figure 4 Northern blot of thermogenin mRNA levels in cultured brown adipocytes

After 7 days in culture, cells were pretreated for 24 h with noradrenaline (24 h NE). The culture medium was then discarded and replaced either with fresh culture medium alone (– NE) or with fresh culture medium plus different treatments: actinomycin (1 μg/ml) (+ Act), cycloheximide (50 μM) (+ CH), noradrenaline (1 μM) (+ NE), actinomycin plus noradrenaline (+ Act + NE), or cycloheximide plus noradrenaline (+ CH + NE). Cells were harvested at time zero (0 h) after the pretreatment period and after 10 h of the different treatments. Samples (5 μg) of total RNA were analysed as described in the Materials and methods section; the insert shows the ethidium bromide-stained RNA revealed by u.v. light. A positive control for thermogenin mRNA (brown-fat RNA isolated from mice exposed to 4 °C for 24 h) (+) and a negative control (brain RNA isolated from the same mice) (–) were included. RNA from untreated cells on day 8 was also analysed (C).

Table 2 Comparison of thermogenin mRNA levels after 10 h of different treatments

Cultured cells were grown and treated as in Figure 4. After the 24 h noradrenaline pretreatment period, the culture medium was discarded and replaced either with fresh culture medium alone or with fresh culture medium plus different treatments as described in the legend to Figure 4. The mean yield of RNA obtained after the different treatments varied between 73 and 137% of that obtained after 24 h noradrenaline pretreatment, but, except for an increase (to 112%; $P < 0.05$) after continued noradrenaline addition, these variations were not significantly different from the yield after the 24 h pretreatment period. The mean thermogenin mRNA level obtained at the end of the 24 h noradrenaline pretreatment period (quadruplicate wells) was in each experiment set to 100% and the values after 10 h treatment (duplicate wells) were expressed relative to this. Values are means ± S.E.M. of 3 independent experiments: * indicates that, by two-way analysis of variance (with duplicate wells) of the result of each of these treatments versus no noradrenaline (– NE), a significant effect of treatment was found ($P < 0.05$ or better).

Treatment	Thermogenin mRNA level (% of initial)
(24 h NE pretreatment)	(*100*)
+ NE	90 ± 12*
– NE	13 ± 0
+ Act	32 ± 8*
Act + NE	52 ± 14*
+ CH	25 ± 7*
+ CH + NE	95 ± 16*

of an mRNA species coding for a protein necessary for rapid degradation, we used the protein-synthesis inhibitor cycloheximide. We used cycloheximide at a concentration which had previously been shown to block thermogenin synthesis in differentiated brown adipocytes in culture [36]; this concentration of cycloheximide had no visible effect on cell appearance, and the yield of RNA after 10 h with this cycloheximide concentration was not statistically different from the initial yield (cf. legend to Table 2). In other cell-culture systems, this concentration of cycloheximide has been demonstrated to inhibit protein synthesis without showing acute cell toxicity [37].

After the 24 h noradrenaline pretreatment period, the cells were treated either with actinomycin or with cycloheximide. The thermogenin mRNA levels were determined 10 h later (Figure 4 and Table 2). As shown, the cessation of adrenergic stimulation again led to a rapid degradation of thermogenin mRNA. The presence of actinomycin again had a stabilizing effect. Furthermore, the presence of cycloheximide led to a similar stabilization of thermogenin mRNA as did actinomycin. It was therefore concluded that a short-lived or induced proteinaceous factor was responsible for the rapid component of the degradation of thermogenin mRNA observed upon cessation of adrenergic stimulation.

Effect of noradrenaline on thermogenin mRNA stability

As it could be inferred from previous experiments *in vivo* with cold-exposed mice [19] that noradrenaline could increase the turnover of thermogenin mRNA, we investigated the effect of noradrenaline on thermogenin mRNA stability in the cell culture system. After the 24 h pretreatment period, the cells were treated either with actinomycin alone or with actinomycin plus continued noradrenaline, and the thermogenin mRNA levels were then determined.

In cells which had received actinomycin alone, thermogenin mRNA levels declined with a half-life of 6.1 h (Figure 5, □). However, in cells which had received both actinomycin and noradrenaline, the half-life of thermogenin mRNA became longer: nearly a doubling to 11.6 h in Figure 5 (■), and a mean value of 10.1 h was obtained in 3 experiments performed in this way (Table 1) (cf. also the independent experiments in Figure 4 and Table 2). Based on two-way analysis of variance of the 4 independent experiments (Figure 5 plus Table 2) with duplicate wells at the 9–10 h time point, the ability of noradrenaline to

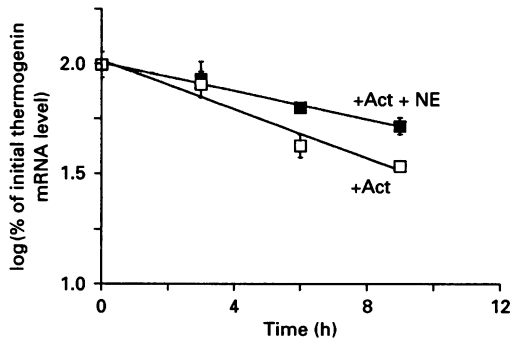

Figure 5 Effect of noradrenaline on thermogenin mRNA stability in the absence of gene transcription

After the 24 h noradrenaline pretreatment period, the culture medium was discarded and replaced either with fresh culture medium plus 1 μg/ml actinomycin (Act) (□) or with fresh culture medium plus actinomycin and noradrenaline (NE) (1 μM) (■). Analysis and representation of results were as in Figure 1. In the presence of actinomycin, the half-life was 6.1 h ($r = 0.93$); in the presence of actinomycin and noradrenaline, the half-life was 11.6 h ($r = 0.94$).

decrease the degree of degradation was found to be highly significant ($P < 0.001$) (the thermogenin mRNA levels at this time point were $38 \pm 6\%$ without noradrenaline and $54 \pm 10\%$ with noradrenaline).

Thus, noradrenaline in this system *in vitro* influenced thermogenin mRNA stability in a way conforming to the general idea that the agent inducing gene expression also stabilizes the mRNA [1]. This result therefore indicates either that the destabilization of thermogenin mRNA previously observed *in vivo*, during cold exposure of mice [19], is not due to a direct effect of noradrenaline on the brown-fat cells themselves, or that the cells differentiated in culture respond differently from those differentiated *in situ*.

DISCUSSION

We have here investigated the stability of thermogenin mRNA in brown adipocytes differentiated in culture. We infer from our results that a factor is induced in the cells after cessation of adrenergic stimulation, and that it is this factor which is responsible for the rapid component of thermogenin mRNA degradation. We also observed that, in the absence of gene transcription, noradrenaline was able to stabilize thermogenin mRNA.

Thermogenin mRNA stability

The half-life of thermogenin mRNA observed, after cessation of adrenergic stimulation of the cultured brown adipocytes, was very short, only 2.7 h (Table 1). This very rapid breakdown may be contrasted with the half-life in the parallel physiological situation, the cessation of cold exposure. In this condition, thermogenin mRNA half-life has been estimated to be 10 h [19] or about 7 h [38] in mice, and 7–11 h in rats [18]. There is no simple explanation for the much more rapid thermogenin mRNA degradation *in vitro* observed here.

If the level of thermogenin mRNA is the major determinant of the rate of thermogenin synthesis, the changes in mRNA levels observed here should precede those occurring in the level of thermogenin itself [2]. In agreement with this, it may be noted that, already during the 24 h noradrenaline pretreatment period, the thermogenin mRNA level had reached a steady state (Figure 1). Under nearly identical conditions, specific thermogenin levels continued to rise for the first 36 h of noradrenaline treatment, only thereafter stabilizing [36]. Thus, as expected, in the presence of noradrenaline, thermogenin mRNA reaches a new steady-state level before the new steady-state protein level is reached. Similar observations on the temporal correlation between induced thermogenin mRNA and protein levels have been made *in vivo*, during cold exposure of mice [24].

The half-life of thermogenin mRNA observed here after cessation of adrenergic stimulation (2.7 h) was much shorter than the half-life of thermogenin itself, which was about 20 h under nearly identical conditions [36]. Thus, when the differentiated cells are no longer adrenergically stimulated, there is a rapid disappearance of thermogenin mRNA, leading eventually to disappearance of thermogenin itself. A similar relationship has been observed *in vivo*, during re-acclimation of cold-exposed mice to warm conditions, where a selective degradation of thermogenin mRNA is initially observed [38].

Thus, in principle, there is agreement between the observations *in vivo* and *in vitro* that changes in thermogenin mRNA levels precede changes in thermogenin levels, implying that thermogenin mRNA determines final thermogenin levels.

Stabilization of thermogenin mRNA by transcriptional and translational blockade

We observed that both transcriptional and translational blockade stabilized thermogenin mRNA. The fact that such treatments stabilized, rather than destabilized, thermogenin mRNA would indicate that gene products are necessary for the degradation of the mRNA, rather than for its protection against degradation.

Stabilization of thermogenin mRNA by transcriptional blockade has previously been observed in experiments *in vivo*: when cold-exposed mice were transferred to a warm environment, the half-life of the mRNA was 10 h, and this was increased to 18 h in animals pretreated with actinomycin [19]. Although the half-lives *in vitro* measured here were shorter than those obtained *in vivo*, the relative effect of actinomycin was the same *in vitro* and *in vivo*. A stabilizing effect of actinomycin under these conditions is not unique for thermogenin mRNA; similar results were obtained for lipoprotein lipase in rat brown adipose tissue [39]. Stabilizing effects of actinomycin have also been observed in other systems, for mRNAs coding for transferrin [40], creatine kinase [41] and neurofilament [42]. Whether the effect of actinomycin indicates that the synthesis of a rapidly decaying transcriptional product is necessary for the degradation process [8] is not clear.

However, the likelihood that the effect of transcriptional blockade may be interpreted in this way, is increased by the fact that, both in the system *in vitro* analysed here, and *in vivo* [19], translational blockade with cycloheximide also stabilized thermogenin mRNA. In general, translational blockade leads to mRNA stabilization [9]. In certain systems, this effect of cycloheximide has been interpreted as indicating that the stability of a given mRNA species is directly related to the rate of translation of this species [9]. However, such an explanation can hardly be relevant for cases such as the present, where transcriptional blockade leads to a stabilization similar to that of translational blockade. Thus, the simplest explanation for the results reported here would be that the effect on thermogenin mRNA stability of either transcriptional or translational blockade is due to cessation of synthesis of an induced (or short-lived) proteinaceous compound, the nature of which is unknown. The kinetics would seem to favour the idea that this compound is induced upon cessation of adrenergic stimulation.

Effect of noradrenaline on thermogenin mRNA stability

We found that noradrenaline, which is the agent that induces thermogenin gene expression, also stabilized thermogenin mRNA. Thus, in this system *in vitro*, the hormonal regulation of thermogenin mRNA stability conforms to the pattern that the inducing hormone also stabilizes the induced mRNA species [1]; a similar observation has recently been made concerning malic enzyme gene induction and mRNA stabilization by triiodothyronine and insulin in the same experimental system [43].

In vivo, as an effect of cold exposure, thermogenin mRNA is destabilized [19]. As pointed out in the Introduction, it has been concluded from experiments *in vitro* that most of the effects of cold exposure on brown-fat cells *in situ* are due to the intensive noradrenaline stimulation of the cells occurring during cold exposure. The fact that noradrenaline was not able to destabilize thermogenin mRNA in the cell-culture system would therefore indicate either that, in this particular aspect, the cultured brown-fat cells are significantly different from brown-fat cells differentiated *in situ*, or that unidentified factors are involved in the regulation of thermogenin mRNA stability. In the latter case, the difference between the short half-life of thermogenin mRNA observed here after cessation of adrenergic stimulation (2.7 h)

and the long half-life observed after cessation of cold stimulation of mice (10 h) [19] may be taken as an indication that it is a stabilizing factor which is missing in the cell-culture system.

Conclusion

The present experiments indicate that as a response to cessation of adrenergic stimulation, an enhanced degradation of thermogenin mRNA occurs; this is apparently due to the induction of a proteinaceous compound, probably a nuclease. Such a mechanism would enable a rapid adjustment to physiological conditions where the demand for brown-adipose-tissue thermogenesis is decreased. Experiments *in vivo* have indicated that such an enhanced degradation may be occurring during reacclimation of cold-exposed animals to warm conditions and involve mRNAs coding for gene products of major significance for thermogenesis [19,39]. An identification of such a physiologically induced nuclease would be of interest for the understanding of the cellular regulation of mRNA levels (and thus of gene expression), as would be the identification of the information in the target mRNAs which makes them susceptible to the action of this mechanism.

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REFERENCES

1 Shapiro, D. J., Blume, J. E. and Nielsen, D. A. (1987) Bioessays **6**, 221–226
2 Hargrove, J. L. and Schmidt, F. H. (1989) FASEB J. **3**, 2360–2370
3 Bernstein, P. and Ross, J. (1989) Trends Biochem. Sci. **14**, 373–377
4 Brawerman, G. (1989) Cell **57**, 9–10
5 Atwater, J. A., Wisdom, R. and Verma, I. M. (1990) Annu. Rev. Genet. **24**, 519–541
6 Nielsen, D. A. and Shapiro, D. J. (1990) Mol. Endocrinol. **4**, 953–957
7 Sachs, A. (1990) Curr. Opin. Cell Biol. **2**, 1092–1098
8 Hentze, M. W. (1991) Biochim. Biophys. Acta **1090**, 281–292
9 Peltz, S. W. and Jacobson, A. (1992) Curr. Opin. Cell Biol. **4**, 979–983
10 Klaus, S., Casteilla, L., Bouillaud, F. and Ricquier, D. (1991) Int. J. Biochem. **23**, 791–801
11 Nedergaard, J. and Cannon, B. (1992) in New Comprehensive Biochemistry, vol. 23: Molecular Mechanisms in Bioenergetics (Ernster, L., ed.), pp. 385–420, Elsevier, Amsterdam
12 Bouillaud, F., Ricquier, D., Mory, G. and Thibault, J. (1984) J. Biol. Chem. **259**, 11583–11586
13 Ricquier, D., Mory, G., Bouillaud, F., Thibault, J. and Weissenbach, J. (1984) FEBS Lett. **178**, 240–244
14 Bouillaud, F., Ricquier, D., Thibault, J. and Weissenbach, J. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 445–448
15 Jacobsson, A., Stadler, U., Glotzer, M. A. and Kozak, L. P. (1985) J. Biol. Chem. **260**, 16250–16254
16 Ricquier, D., Bouillaud, F., Toumelin, P., Mory, G., Bazin, R., Arch, J. and Penicaud, L. (1986) J. Biol. Chem. **261**, 13905–13910
17 Jacobsson, A., Nedergaard, J. and Cannon, B. (1986) Biosci. Rep. **6**, 621–631
18 Reichling, S., Ridley, R. G., Patel, H. V., Harley, C. B. and Freeman, K. B. (1987) Biochem. Biophys. Res. Commun. **142**, 696–701
19 Jacobsson, A., Cannon, B. and Nedergaard, J. (1987) FEBS Lett. **224**, 353–356
20 Silva, J. E. (1988) Mol. Endocrinol. **2**, 706–713
21 Freeman, K. B., Heffernan, M., Dhalla, Z. and Patel, H. V. (1989) Biochem. Cell Biol. **67**, 147–151
22 Obregon, M. J., Jacobsson, A., Kirchgessner, T., Schotz, M. C., Cannon, B. and Nedergaard, J. (1989) Biochem. J. **259**, 341–346
23 Rehnmark, S., Bianco, A. C., Kieffer, J. D. and Silva, J. E. (1992) Am. J. Physiol. **262**, E58–E67
24 Jacobsson, A., Mühleisen, M., Cannon, B. and Nedergaard, J. (1994) Am. J. Physiol., in the press
25 Tvrdik, P., Kuzela, S. and Houstek, J. (1992) FEBS Lett. **313**, 23–26
26 Rehnmark, S., Néchad, M., Herron, D., Cannon, B. and Nedergaard, J. (1990) J. Biol. Chem. **265**, 16464–16471
27 Niijima, A., Rohner-Jeanrenaud, F. and Jeanrenaud, B. (1984) Am. J. Physiol. **247**, R650–R654
28 Young, J. B., Saville, E., Rothwell, N. J., Stock, M. J. and Landsberg, L. (1982) J. Clin. Invest. **69**, 1061–1071
29 Nedergaard, J. and Lindberg, O. (1982) Int. Rev. Cytol. **74**, 187–286
30 Bronnikov, G., Houstek, J. and Nedergaard, J. (1992) J. Biol. Chem. **267**, 2006–2013
31 Rehnmark, S., Kopecky, J., Jacobsson, A., Néchad, M., Herron, D., Nelson, B. D., Obregon, M. J., Nedergaard, J. and Cannon, B. (1989) Exp. Cell Res. **182**, 75–83
32 Néchad, M., Nedergaard, J. and Cannon, B. (1987) Am. J. Physiol. **253**, C889–C894
33 Rehnmark, S., Antonson, P., Xanthopoulos, K. G. and Jacobsson, A. (1993) FEBS Lett. **318**, 235–241
34 Kohno, K., Hamanaka, R., Abe, T., Nomura, Y., Morimoto, A., Izumi, H., Shimizu, K., Ono, M. and Kuwano, M. (1993) Exp. Cell Res. **208**, 498–503
35 Herron, D., Rehnmark, S., Néchad, M., Loncar, D., Cannon, B. and Nedergaard, J. (1990) FEBS Lett. **268**, 296–300
36 Puigserver, P., Herron, D., Gianotti, M., Palou, A., Cannon, B. and Nedergaard, J. (1992) Biochem. J. **284**, 393–398
37 Kaufmann, S. H. (1991) Cancer Res. **51**, 1129–1136
38 Patel, H. V., Freeman, K. B. and Desautels, M. (1987) Biochem. Cell Biol. **65**, 955–959
39 Mitchell, J. R. D., Jacobsson, A., Kirchgessner, T. G., Schotz, M. C., Cannon, B. and Nedergaard, J. (1992) Am. J. Physiol. **263**, E500–E506
40 Müllner, E. W. and Kühn, L. C. (1988) Cell **53**, 815–825
41 Pontecorvi, A., Tata, J. R., Phyillaier, M. and Robbins, J. (1988) EMBO J. **7**, 1489–1495
42 Schwartz, M. L., Shneidman, P. S., Bruce, J. and Schlaepfer, W. W. (1992) J. Biol. Chem. **267**, 24596–24600
43 García-Jimenez, C., Hernández, A., Obregon, M. J. and Santisteban, P. (1993) Endocrinology (Baltimore) **132**, 1537–1543

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