Difference in induction of uncoupling protein genes in adipose tissues between young and old rats during cold exposure

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Received 27 July 1999

Abstract Induction of uncoupling protein (UCP) genes in adipose tissues from young and old rats exposed to cold was compared. UCP1 mRNA expression in brown adipose tissue (BAT) was enhanced in both young and old rats after cold exposure, but the expression was downregulated at 72 h after the exposure only in the young rats. The UCP2 gene was induced notably in BAT of young rats instead of the downregulation of the UCP1 gene, whereas the induction in old rats was almost blunted. The pattern of UCP3 expression was similar to that of UCP1 expression in each group. The effect of cold exposure on the expression of UCP2 genes was also observed in white adipose tissue from the young rats. These results indicate a change in induction of UCP genes in adipose tissues with aging.

Key words: Uncoupling protein; Gene expression; Adipose tissue; Aging; Cold

1. Introduction

Thermogenesis in mammals is an essential physiological function to maintain the body temperature, to dissipate excess energy, or in the defense mechanism against infection. Mitochondrial uncoupling proteins (UCPs), which have a potential to generate heat by uncoupling oxidative phosphorylation, appear to play a crucial role in thermogenesis [1]. At present, three subtypes of UCP, which share over 55% homology with each other, are known [2–5]. UCP1 is expressed exclusively in brown adipose tissue (BAT). The recently cloned UCP2 and UCP3 are expressed in many tissues including BAT and white adipose tissue (WAT) and dominantly in muscle and BAT, respectively. Many studies have described that UCPs are involved in the control of body temperature and the regulation of energy balance. UCP1 is indispensable for cold tolerance [6] and is involved in the mechanism regulating body weight [7], at least in rodents. UCP2 and UCP3 have been implicated in lipid utilization [8] or antioxidant mechanism [9]; however, their relevance to obesity and diabetes has been argued [10]. Boyer et al. [11] suggested a potential role of UCP2 and UCP3 in thermal homeostasis during hibernation in a study using ground squirrels. The gene expression of these UCPs seems to be regulated differentially [11–13]. However, the differences in the roles of three UCPs and the correlation of their gene expression remain to be elucidated.

The capacity for thermogenesis tends to be attenuated with age [14]. A simple but good way to assess thermogenic ability in individuals is to assess the response to cold. Using this method, an age-related decline in cold-induced thermogenesis (CIT) has been demonstrated by several groups, in which a decreased BAT thermogenic capacity and the inhibited UCP activity were observed in old animals [14]. Although of the three UCP subtypes expressed in BAT UCP1 is most abundant and responsible for CIT, the roles of UCP2 and UCP3 in CIT are still not clear, and it is not known if the induction of UCP genes in cold changes with age. The prevalence of obesity also increases with age especially in Western countries [16]. The adiposity (WAT mass) increases in laboratory animals with age even under the condition with common diet ad libitum [17]. They usually spend a sedentary life in thermostatically controlled rooms, quite similar to our modern life style. So the susceptibility to obesity in inactive old animals seems to increase with the attenuation of thermogenic ability.

In the present study, we examined whether there are differences in the induction of the three types of UCP in fat tissues between young and old rats during cold exposure and whether there is a connection between the change in UCP expression, diminished thermogenic ability and obesity with age.

2. Materials and methods

2.1. Animals

Male Wistar strain rats were obtained from Japan SLC at 7 weeks of age. The rats were housed in groups of 3–5 under barrier conditions and reared at 25°C under artificial lighting for 12 h with standard laboratory diet and tap water ad libitum. The rats were used either at 10–14 weeks of age (249 ± 12 g, $n = 24$) or at 26 months of age (419 ± 5 g, $n = 18$). Only rats without overt signs of disease were used; however, one old rat died after 24 h cold exposure. The body temperature of rats was measured by an electronic thermistor equipped with a rectal probe before the experiment of cold exposure. The body temperature of young and old rats under the condition of room temperature was 38.0 ± 0.2 and 37.1 ± 0.3 (means ± S.D., $n = 6$), respectively. We did not measure the body temperature during cold exposure to avoid the stress of handling which could induce an increase in body temperature.

In the present experiments the young and old rats were maintained individually at 4°C for 0, 3, 24, or 72 h (4–6 mice for each time point). After the change in body weight was determined, interscapular BAT and epididymal WAT were collected in the cold and used for total RNA preparation. All rats were cared for according to institutional guidelines.

2.2. RNA analysis

Total RNA (20 μg), isolated from BAT and WAT using the acidic guanidinium isothiocyanate method [18], was analyzed by Northern blots as described previously [6]. Blots were hybridized with probes (labeled with [^{32}P]dCTP) for the mRNAs of UCP1 [15], UCP2 [6], UCP3, leptin, and 18S ribosomal RNA [6]. The UCP3 and leptin probes were produced from positions 427 to 726 of the rat sequence (accession number U92069) and from positions 49 to 564 of the mouse sequence (accession number U18812), respectively, by PCR. Hybridization signals were quantified by the Fuji Bioimage Analyzer. The statistical significance of the data was assessed by ANOVA (Fisher's PLSD, Statview 4.1).

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PII: S0014-5793(99)01143-6

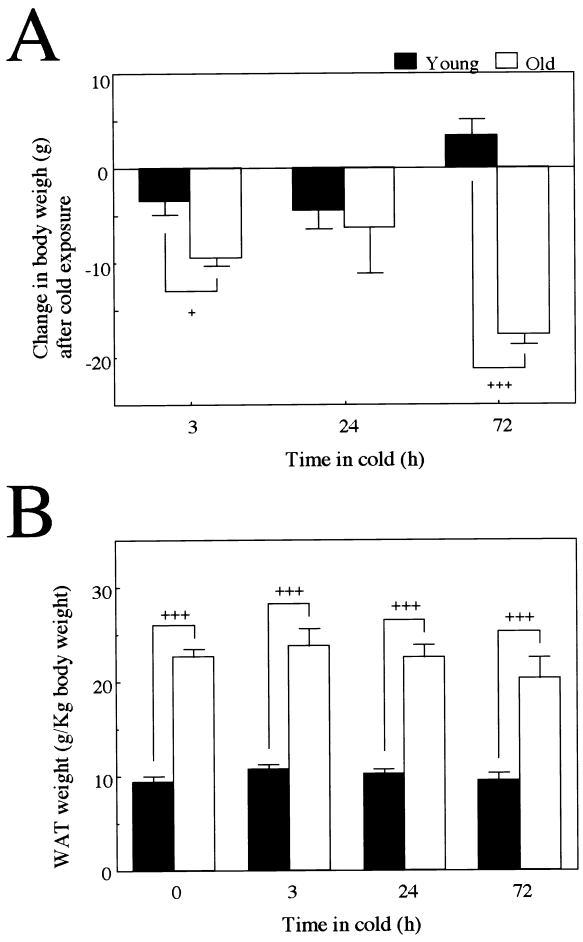

Fig. 1. Changes in body (A) and WAT (B) weights of the young and old rats during cold exposure. Data are expressed as means ± S.E.M. ($n = 4$–6). $^{+}P < 0.05$, $^{+++}P < 0.001$ vs. young rats at the same time point.

3. Results

Fig. 1A shows the change in body weight during cold exposure. The old rats had continuous body weight loss in the cold, whereas the weight in the young rats recovered to the level before the exposure after 72 h, indicating that the old rats were intolerant to cold and the young rats were cold-resistant. A marked increase in WAT mass was observed in the old rats compared to the young rats (Fig. 1B), although the WAT mass in epididymal depot did not change significantly during cold exposure even in the old rats in spite of the body weight loss. The body temperature at room temperature was lower ($P = 0.0006$) in the old rats than in the young rats.

We next examined UCP and Lep gene expression in BAT and WAT during cold exposure. There were no big differences in the mRNA levels of UCP1, UCP2, and UCP3 in BAT from young and old rats maintained at 25°C. However, it was found that there were great differences in the induction of UCP genes in BAT during cold exposure between young and old rats (Fig. 2). UCP1 mRNA level was increased rapidly in BAT from both groups as expected, whereas the expression was downregulated markedly to 28% of the control level at 72 h after cold exposure in the young rats. In the old rats, compared to the peak value at 24 h, the UCP1 mRNA level was decreased at 72 h after cold exposure, but the level was still higher (2.2-fold) than the control. In contrast to the downregulation of UCP1 in the young rats, UCP2 was gradually but notably induced (seven-fold of the control at 72 h after cold exposure). The UCP2 mRNA level in BAT at 72 h was comparable to the level in WAT. On the other hand, there was no significant change in UCP2 mRNA level in the old rats until 72 h after cold exposure. The UCP3 expression in BAT of the young rats was downregulated in the cold, which was reciprocal to the UCP2 expression. In the old rats, however, the pattern of UCP3 expression was similar to the pattern of UCP1 expression and peaked at 24 h (166% of the control level). The leptin mRNA level in BAT was higher in the old rats than in the young rats and the expression was reduced greatly. Our data of UCP1 and Lep gene expression in BAT indicate that these genes are not always regulated reciprocally, as distinct from the results of Cancello et al. [19].

In WAT, the expression of the UCP2 gene was higher in the young rats than in the old rats but ob gene expression was not distinct between young and old rats (Fig. 3). The UCP2 mRNA level in the old rats was much lower than the level in the young rats. The UCP2 expression in the young rats was significantly increased up to 132% of the control level at 72 h after cold exposure, whereas there was no change in the old rats. The leptin mRNA level was reduced to about 40% of the control level after the exposure in both young and old rats although there was no significant change in WAT mass. These results indicate that the effect of cold exposure on UCP2 and leptin expression in BAT and WAT was alike in each group.

4. Discussion

It has been demonstrated that old animals including humans are intolerant to cold because of a decline in thermogenic ability, especially in CIT, with age [14]. The lack of CIT in old animals appears to originate from the reduced activity of UCP, probably UCP1 in BAT, because UCP1 is most responsible for CIT [6]. In the present study the old rats had continuous weight loss during cold exposure, suggesting hypoactive thermogenesis in the old rats since rats displaying spontaneous rapid weight loss have significant hypothermia during cold exposure [20]. The weight loss could indicate a more inefficient mechanism of thermogenesis because UCP1-derived thermogenesis is reduced.

We have previously demonstrated that BAT thermogenic activity is markedly reduced in spite of substantial UCP1 expression [15]. As expected, the mRNA level of UCP1 in BAT was increased by cold exposure in the old rats like in the young rats, indicating that the signaling mechanism for UCP1 induction was intact in the old rats. The comparison of UCP1 expression patterns between the young and old rats suggests a slow adjustment to changing temperature in the old rats. The high level of UCP1 mRNA was maintained in the old rats until 72 h after cold exposure, whereas UCP1 gene expression in the young rats was downregulated greatly at 72 h after cold exposure, suggesting a greater need for UCP1 thermogenesis in old rats. The downregulation of the UCP1 gene in the cold was unexpected because similar results have not been reported previously. Interestingly, we found a notable induction of the UCP2 gene in the young rats, which was regulated inversely to UCP1 and UCP3 expression. Boss et al.

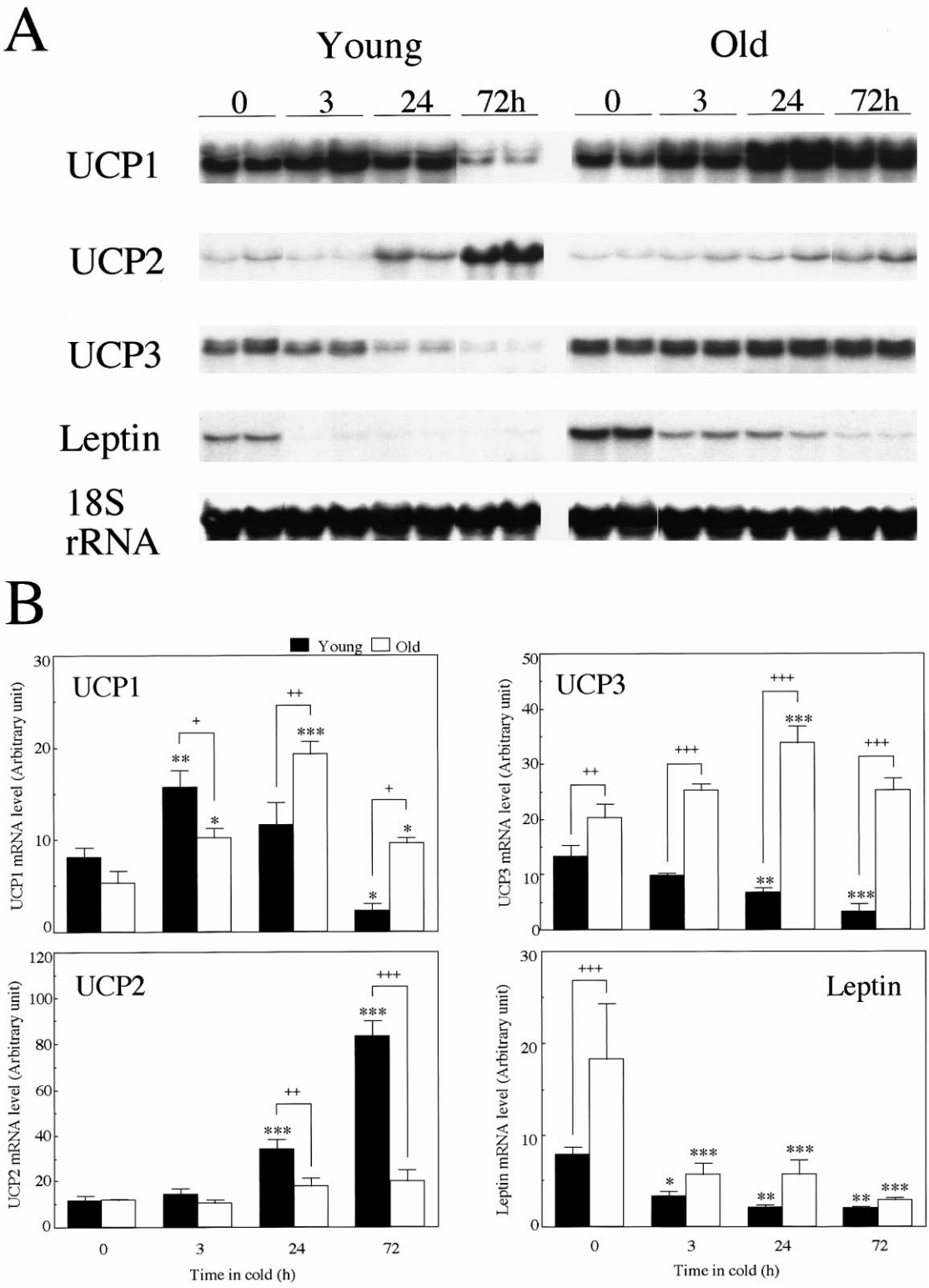

Fig. 2. Induction of UCPs and leptin genes in BAT during cold exposure. Young and old rats were exposed to the cold for the indicated times. A: Northern blots using total RNA from BAT were hybridized with the probes for UCP1, UCP2, UCP3, leptin and 18S rRNA as described in Section 2. B: mRNA levels of UCP1, UCP2, UCP3 and leptin in BAT of young and old rats. The results were normalized by 18S rRNA levels. Data are expressed as means ± S.E.M. ($n = 4$–6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control (0 h) in each group. $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ vs. young rats at the same time point.

[21] have observed 2.4-fold increase of UCP2 mRNA level but no downregulation of the UCP1 gene in BAT of 7 week old Sprague-Dawley rats after 48 h cold exposure. Their data appear to be similar to the data after 24 h cold exposure in our study, although we do not know the expression of UCP genes at the same time point (48 h after cold exposure). The gene expression of UCPs could be varied by differences of experimental conditions used (e.g. the strain and age of ani-

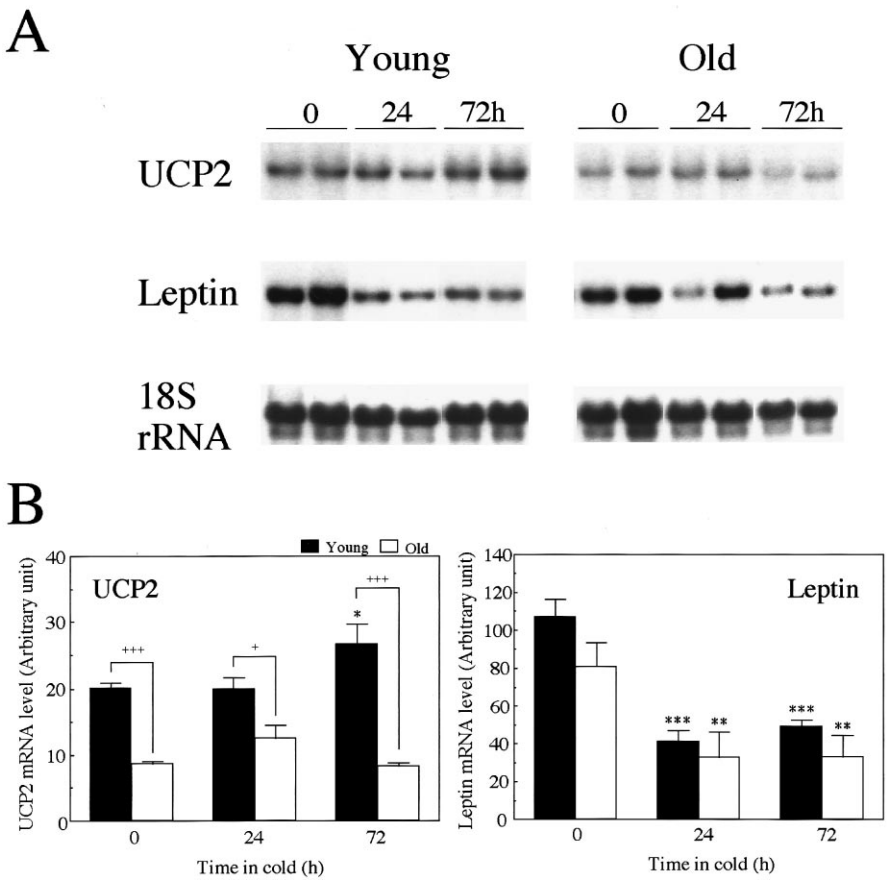

Fig. 3. Induction of UCP2 and leptin genes in WAT during cold exposure. Young and old rats were exposed to the cold for the indicated times. A: Northern blots using total RNA from WAT were hybridized with the probes for UCP2, leptin and 18S rRNA as described in Section 2. B: mRNA levels of UCP2 and leptin in WAT of young and old rats. The results were normalized by 18S rRNA levels. Data are expressed as means ± S.E.M. ($n = 4$–6). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control (0 h) in each group. $^{+}P < 0.05$, $^{+++}P < 0.001$ vs. young rats at the same time point.

mals). The steady-state level of UCP2 mRNA at room temperature is low and the induction was almost blunted in the old rats. Differences in adaptability to cold between young and old rats are also suggested from the viewpoint of UCP expression.

UCP3 is expressed at a substantial level in BAT of rats. Previous studies using young animals have demonstrated an increase of UCP3 mRNA level after 7 days of cold exposure [12] or no effect by 5 h acute exposure to cold [22]. In our study the regulation of the UCP3 gene in BAT during cold exposure for 3 days was completely opposite between the young and old rats. The expression in the young rats was gradually attenuated in contrast to the induction of the UCP2 gene, suggesting a relay of thermogenic function or a change in energy homeostasis if they have different roles in energy metabolism. Energy expenditure might shift to weight control from maintenance of body temperature in young rats because the decrease in body weight after cold exposure was recovered at 72 h after cold exposure in the young rats (but not in the old rats). In contrast, the steady-state level of UCP3 mRNA before cold exposure was higher in the old rats than in the young rats and the expression in the old rats was further enhanced after cold exposure in a similar manner to UCP1 expression. Although we did not determine UCP3 expression in muscle, UCP3 could have participated in CIT in the old rats to supply a need for heat.

To date the linkage of UCPs to obesity or diabetes has been demonstrated by a number of investigations [4,7,10]. The studies using UCP1 transgenic mice suggest that animals with reduced UCP1 activity have a high susceptibility to obesity as well as intolerance to cold [7]. An increased level of UCP2 mRNA is observed in WAT of ob/ob and db/db mice [3], while neuropeptide Y-Y1 receptor-deficient mice with mild hyperinsulinemia have lowered UCP2 expression in WAT and develop mild obesity [23]. A high fat diet increases the UCP2 mRNA level in WAT in obesity-resistant KsJ and A/J mice but not in obesity-prone C57BL/6J mice [4,24]. Obese animals often have a lower core temperature than lean animals [25]. In the present study the old rats had increased WAT weight/body weight, lower body temperature, and presumably mild hyperinsulinemia as previously reported [15], compared to the young rats, suggesting that the old rats were mildly obese. The accumulation of body fat may improve thermoinsulation but lower the set point of the body thermostat so that it leads to a further decrease in heat production. The leptin mRNA level in WAT from the old rats was not distinct from that in the young rats, although the WAT weight was much higher in the old rats than in the young rats. These results are consistent with the study of Li et al. [16] that adiposity increases with age but the leptin mRNA level in epididymal WAT was unchanged with age. The leptin expression was reduced in WAT as well as in BAT in both young and old rats after

cold exposure, suggesting stimulation of food intake. Because food intake in the cold is increased after an initial fall on the first day of cold exposure (Yamashita, unpublished data), the increase in UCP2 mRNA level in the fat tissues in the young rats could be associated with an increase of food intake and the recovery of body weight. Considering the differences in the expression pattern of the UCP2 gene and the mRNA level in WAT between the young and old rats, it is possible that a failure of UCP2 induction and the reduced level of UCP2 in old rats increase the susceptibility to obesity. Because mice lacking UCP1 with a compensated induction of the UCP2 gene in BAT are sensitive to acute cold exposure [6], the role of UCP2 may be greater in diet-related regulation of energy balance than in CIT, although the involvement of UCP2 in adaptive thermogenesis in a cold environment, suggested by Carmona et al. [22], cannot be denied.

Thus, our data indicate differences in the induction of UCP genes in fat tissues and a difference of dependence on UCP subtype in the cold between young and old rats. In addition to UCP inactivation, a failure of UCP2 induction may be concerned in the decline of thermogenic ability with aging. It is likely that a decline of UCP function in the aging process with reduced physical activity increases the cold sensitivity and susceptibility to obesity; however, we need further study to elucidate the genetic and environmental factors raising the transcriptional change and inactivation of UCPs with aging.

Acknowledgements: This work was supported in part by The Naito Foundation (H.Y.). We are grateful to L.P. Kozak for kindly providing the UCP2 probe and for valuable comments. We also thank T. Mizuno and T. Miura for their technical assistance.

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