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MUSCLE PROTEIN SYNTHESIS RATES DURING TEMPERATURE ACCLIMATION IN A EURYTHERMAL (*CYPRINUS CARPIO*) AND A STENOTHERMAL (*SALMO GAIRDNERI*) SPECIES OF TELEOST

BY P. T. LOUGHNA* AND G. GOLDSPINK*

Muscle Research Unit, Department of Zoology, University of Hull, Hull, England HU6 7RX

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SUMMARY

Rates of protein synthesis were measured in the white myotomal muscle of carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*) at a number of environmental temperatures before and after they had spent an acclimatory period at these temperatures. For carp previously kept at a mid-range temperature of 15 °C the rates of muscle protein synthesis showed a Q_{10} of 3·07 over the range of 10–25 °C. Acclimation to low temperatures resulted in the rate of protein synthesis being elevated whilst acclimation to high temperatures resulted in the depression of protein synthesis rate. Rainbow trout also initially maintained at 15 °C showed an initial Q_{10} of 3·57 between 5 and 20 °C. In this species, however, there was no significant difference in protein synthesis rates after being kept for 1 month at any given temperature within this temperature range. In the carp where acclimatory changes were recorded, it was found that these changes occurred at a faster rate at 25 °C than at 10 °C. The ability of the carp to modify its protein metabolism in this way is discussed as a strategy for reducing temperature effects upon growth and muscle function.

INTRODUCTION

Fish, which are probably the only true obligate vertebrate poikilotherms, occupy a remarkable range of thermal niches. Adaptation to these niches has taken place over thousands of years and explains the wide diversity of environments now occupied by fish species, ranging from the Antarctic, with temperatures of −1 °C or −2 °C, to hot springs in the Rift Valley and in Arizona, with temperatures of about 40 °C. However, many of these species will be subjected to seasonal and even more acute temperature changes. This raises the question as to

*Present address: Department of Anatomy and Cellular Biology, Tufts University Schools of Medicine and Veterinary Medicine, Boston, MA 02111, U.S.A.

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whether anabolic and catabolic rates are simply a function of temperature or whether mechanisms have evolved to endow some temperature independence.

The respiratory metabolism of a number of cold water species has been found to be significantly greater than would be expected on the basis of rates in temperate and tropical fishes (Scholander, Flagg, Walters & Irvine, 1953; Wohschlag, 1964). These conclusions based upon oxygen consumption results have been criticized due to inherent difficulties in standardizing their activity which is also temperature dependent (Holeton, 1974). Other studies on enzyme systems, however, support the notion of interspecific temperature compensation (Low & Somero, 1974; Borgmann & Moon, 1975; Johnston & Goldspink, 1975). Smith & Haschemeyer (1980) proposed that protein synthetic activity be used as a basis for evaluation of interspecific metabolic adaptation based upon the observation that protein synthesis in mammals bears a constant ratio to basal metabolic rate. They found that Antarctic fish have muscle protein synthesis rates three times greater than would be expected from extrapolation of data from fish from warmer waters.

Many fish species are strictly stenothermal and have little tolerance to temperature change. Other species, however, are subject to large changes in temperature. When these changes occur over a few hours, there is insufficient time for metabolic reorganization and the animal has to tolerate them. Obviously, this does not allow the animal to function optimally for prolonged periods of time (Sidell, Johnston, Moerland & Goldspink, 1983). Seasonal fluctuations in environmental temperature, however, allow eurythermal animals to undergo adaptive compensation. A number of studies have indicated that cold-acclimated animals have higher metabolic rates than warm-acclimated ones when both are measured at the same temperature (Kanungo & Prosser, 1959; Campbell & Davies, 1975). Also, some teleost species are known to be able to alter their myofibrillar system (Johnston, 1979; Penney & Goldspink, 1981), the proportion of fibre types within the myotome (Sidell, 1980) and the way the different types of muscle are recruited in order to maintain locomotory ability when acclimated to lower temperatures (Loughna, Rome & Goldspink, 1983). Although quantitative interspecific comparisons of protein synthesis have been made by Smith & Haschemeyer (1980) as discussed above, very little is known about the effect of temperature within an individual species. There are at present no data available concerning acclimatory changes in protein synthesis or degradation and the only data available for acute temperature effects are upon the liver of the toadfish (Mathews & Haschemeyer, 1978). This study, therefore, is aimed at elucidating acute temperature effects on muscle protein synthesis and determining to what extent these may be modified by acclimatory compensation (seasonal adaptation) in two species of fish.

MATERIALS AND METHODS

Animals

Fish used in this study were rainbow trout (*Salmo gairdneri*) and mirror carp (*Cyprinus carpio*), both obtained from a local fish farm. They were kept in running

fresh water at 15 ± 1 °C for at least 1 month prior to being acclimated to different temperatures, and for at least 2 weeks when used for the time course study for the plasma tyrosine levels and for the leucine incorporation experiments. Throughout this period and while the animals were acclimating, they were kept in a 14 h light: 10 h dark cycle and fed twice daily to satiation with commercial fish diet. Rainbow trout used for the isotope time course study weighed 275–325 g. For the temperature experiments, smaller fish were used (rainbow trout weighed 50–85 g and carp weighed 30–60 g). These fish were sexually immature and no attempt was made to sex individuals.

Measurement of muscle protein synthesis rates

Previously, the constant infusion technique has been used for measuring protein synthesis in fish (Haschemeyer, Persell & Smith, 1979; Haschemeyer & Smith, 1979; Loughna & Goldspink, 1984). However, due to the large number and small size of the fish used in this study, a method involving only a single injection was adopted. This was based on the method originally described by McNurlan, Tomkins & Garlick (1979). Fish were injected intraperitoneally with 1 ml/100 g of animal of tritiated phenylalanine solution at a concentration of 150 mmol l^{-1} and an activity of 10 μCi ml^{-1}. The fish were then left for 6 h in individual containers before being sacrificed and frozen in liquid nitrogen. Tissue was removed while still frozen and rapidly homogenized in two volumes of perchloric acid (PCA). Each sample was then centrifuged at 1500 ***g*** for 10 min. The supernatant was removed and stored at −20 °C. The precipitate was washed several times by resuspending in PCA, centrifuging as previously described and then discarding the supernatant. The washed precipitate was finally hydrolysed in 6 mol l^{-1} hydrochloric acid at 110 °C for 18 h. The final hydrolysate was dried in a vacuum and then 2 ml of citrate buffer (pH 5·5, 0·5 mol l^{-1}) was added. An enzyme suspension was made up to a concentration of 0·5 mg ml^{-1} phenylalanine decarboxylase, 0·5 mg ml^{-1} tyrosine decarboxylase and 1 mg ml^{-1} pyridoxal phosphate in citrate buffer (pH 5·5, 0·5 mol l^{-1}). One millilitre of this suspension was added to the amino acid solution and it was incubated overnight at 55 °C. This process converts 98–100 % of the phenylalanine to β-phenylethylamine and L-tyrosine to tyramine. The previously stored supernatants were thawed and the PCA was neutralized to pH 7·0 by addition of saturated tripotassium citrate solution. These samples were then incubated with the enzyme solution as described for the hydrolysates. β-phenylethylamine was then extracted by adding 1 ml of 6 mol l^{-1} sodium hydroxide solution and shaking with 15 ml of chloroform/heptane (1:3 v/v). The organic layer was removed and discarded. Two more additions of chloroform/heptane were made, followed by shaking and subsequent removal of the organic layer after settling. The tyramine was then extracted from the remaining aqueous residue as described by Garlick & Marshall (1972). Of the final aqueous tyramine solution a 1-ml sample was used for scintillation counting and duplicate samples (1 ml for supernatants, 0·1 ml for hydrolysates) were assayed for tyramine using the fluorimetric method of

Waulkes & Udenfriend (1957). The specific activities of tyramine extracted from the hydrolysate and from the free amino acids were calculated from the amount of tyramine present per unit volume and the disintegrations per minute (d.p.m.) calculated for the same volume. A Packard tricarb scintillation counter was used for measuring counts per minute and from this d.p.m. were calculated using a standard quench correction procedure.

Time course study

Fish to be used in the time course study had dorsal aorta cannulae implanted 48 h prior to injection. Four fish were injected with tritiated phenylalanine. Blood samples of approximately 180 μl were taken at six or seven time points over the 6-h period. The specific activity of tyrosine in the plasma from the injected animals was estimated as previously described.

Leucine incorporation experiment

Ten rainbow trout were injected intraperitoneally with 1 ml/100 g of phenylalanine solution (150 mmol l^{-1}) and ten controls were administered a pulse dose of saline. Both the saline and the phenylalanine solution contained 6 μCi ml^{-1} of [^{14}C]leucine. Five fish from each group were killed after 1 h and another five after 6 h. Approximately 2 g of white epaxial muscle was removed from each fish after it had been killed. This tissue was then homogenized in PCA. It was then centrifuged, the precipitate washed and hydrolysed as described above. [^{14}C]leucine incorporation was measured in a scintillation counter.

Temperature acclimation experiments

Carp were acclimated to 10, 20 and 25 °C and rainbow trout acclimated to 5, 10 and 20 °C. Data concerning rates of protein synthesis in these groups were then compared to data from control groups in which the fish were not given sufficient time to acclimate at the measurement temperature. The control fish, however, were not placed immediately at the measurement temperature as this would have caused undue thermal stress and possibly given rise to erroneous results. All the acclimation and control groups for both species were placed in separate tanks (at 15 °C) 1 week prior to the start of the experiment. The water temperature in the tanks was then changed at a rate of approximately 1 °C per hour until the desired acclimation and measurement temperatures were reached. In the acclimated groups the fish were allowed to stay at this temperature for 1 month plus 24 h whereas the controls were allowed to equilibrate for only 24 h before they were killed for the protein synthesis measurements. Six hours prior to being killed, five fish in each temperature group were injected with labelled amino acid whilst still being maintained at the acclimation/measurement temperatures.

In addition to measuring the effects of acclimation over 1 month (28 days), the time course for changes in protein synthesis was examined for the extreme temperatures in both species. At these temperatures, fish were injected and

killed at 3 days, 7 days and 14 days after reaching the new acclimation temperature.

RESULTS

Time course for amino acid in the blood

The time course for rise of specific activity of radioactively labelled tyrosine, following a single injection for three cannulated fish is shown in Fig. 1. It can be seen that for all three fish specific activity in the plasma increased to a plateau which was maintained fairly constant during the remainder of the 6-h period. Phenylalanine is converted by the liver into tyrosine (Alton-Meister, 1965) after it has been absorbed into the bloodstream from the intraperitoneal cavity. Although in each individual it seems that a fairly steady plateau was achieved with this pulse dose method, the level varied between individuals. This still allows muscle protein synthesis to be calculated for each individual animal using the equation of Garlick, Millward & James (1973). Protein synthesis rate for muscle was obtained from:

$$\mathrm{Sb/Si} = \frac{\mathrm{R} \times [1 - \exp(-\mathrm{Kst})]}{\mathrm{R} - 1 \times [1 - \exp(-\mathrm{RKst})]} \times \frac{-1}{\mathrm{R} - 1},$$

where Ks is the fractional rate of protein synthesis, Sb and Si are the specific activities of tyrosine in the protein pool and free amino acid pool, respectively, and R is the ratio of protein-bound tyrosine to that in the free amino acid pool. It can be seen from Table 1 that the large pulse dose of 'cold' phenylalanine had no significant effect upon the uptake of [^{14}C]leucine into epaxial muscle over a 6-h period (calculated by a Student's *t*-test).

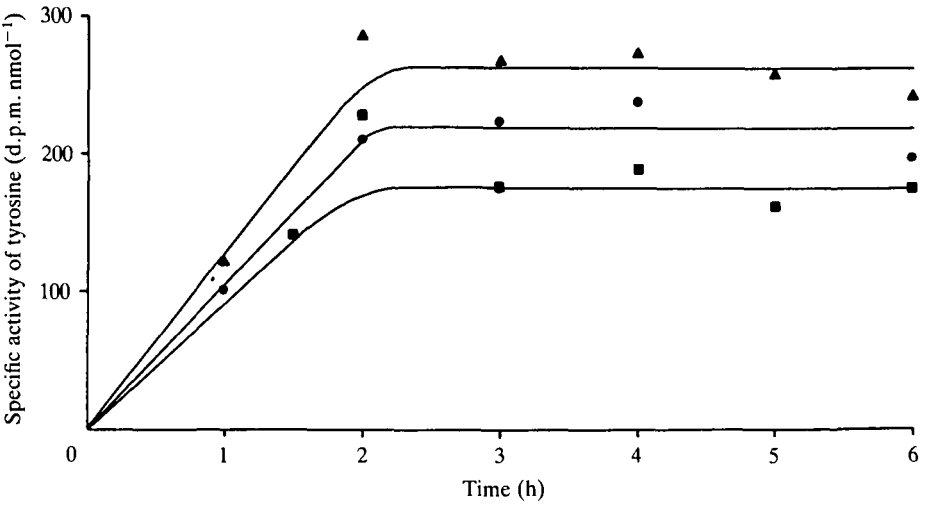

Fig. 1. Time course for specific activity of tyrosine in the plasma of three rainbow trout after an intraperitoneal injection of [^{3}H]phenylalanine.

Table 1. *The effects of an intraperitoneal injection of saline and concentrated phenylalanine solution upon incorporation of [^{14}C]leucine into white muscle protein of* Salmo gairdneri

Time interval after injection (h)	d.p.m. mg^{-1} protein residue		Significance (t-test)
	Saline	Phenylalanine	
1	71·6±27·9	93·2± 36·1	Not significantly different at $P=0·3$
6	689±97·9	594·3±107·4	Not significantly different at $P=0·3$

Values are mean±S.D. for five fish.

Temperature effect on protein synthesis

The temperature dependency of protein synthesis rates in white muscle of carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*) is shown in Fig. 2 for fish acclimated for 24 h and 1 month. In the rainbow trout, at no temperature were the rates of protein synthesis significantly different after 1 month of acclimation when compared to 24 h of exposure at that temperature (calculated by Student's t-test). In the carp, no significant difference was found between the two time periods at 15 °C (the original acclimation temperature) and at 20 °C. However, at 10 °C, rates of white muscle protein synthesis were significantly higher (Student's t-test, $P<0·001$) after a month of acclimation at this temperature when compared to the values obtained after 24 h. The inverse of this occurred at 25 °C in this species and

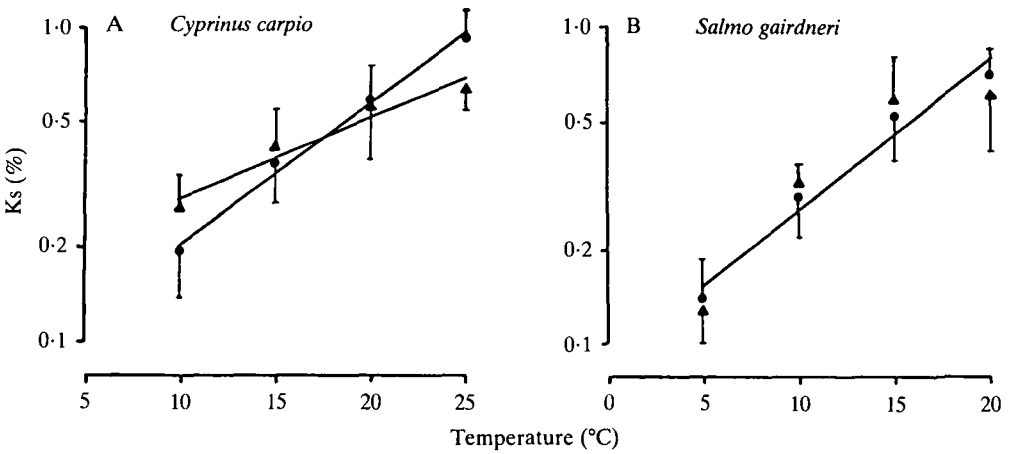

Fig. 2. (A) Arrenhius plots of fractional synthesis rates of white muscle of carp acclimated for 24 h (●) and 1 month (▲). (B) An Arrenhius plot for fractional synthesis rate of white muscle of rainbow trout acclimated for 24 h. Rates of synthesis after 1 month are shown though no regression line is plotted. Values are means of five animals±S.D. Ks, fractional rate of protein synthesis.

1 month of acclimation at this temperature caused a significant reduction in white muscle protein synthesis. The Q_{10} for protein synthesis rate obtained for rainbow trout over the temperature scale 5–20 °C at 24 h was 3·57 which was higher than the Q_{10} obtained for carp of 3·07 over the range 10–25 °C.

Fig. 3 shows the time course for protein synthesis rates at 10 °C, 15 and 25 °C in the carp over the period of 1 month. It can be seen that in warm-acclimated carp, the rate of fall in protein synthesis was quite rapid. In the cold-acclimated fish, there was a fall in protein synthesis over the first 3 days which may possibly be due to reduced feeding during this initial period of cold acclimation. However, rates of synthesis subsequently rose gradually.

DISCUSSION

The method employed in this study to measure rates of muscle protein synthesis involved flooding the amino acid pools of the body tissues of the fish with a pulse dose of tritiated phenylalanine. A rapid flooding of these free amino acid pools, as used by McNurlan *et al.* (1979) and later in numerous other

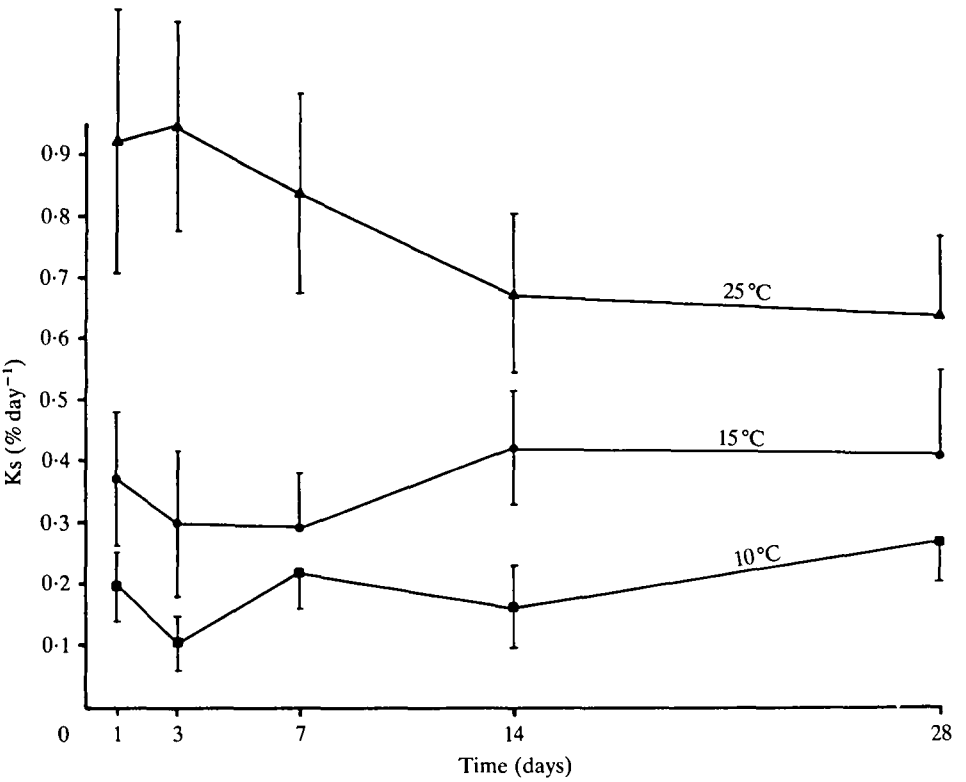

Fig. 3. Time course for temperature compensation in protein synthesis in *Cyprinus carpio* acclimating to 25 °C and 10 °C with control fish at 15 °C. Values are means ±S.D. Ks, fractional rate of protein synthesis.

mammalian studies, cannot be achieved due to the very poor vascularization of the white epaxial muscle and also the low body temperature. The method used in this study, however, is dependent upon the conversion of phenylalanine to tyrosine by phenylalanine 4-hydroxylase in the liver. Thus, the liver is, in effect, acting as an internal constant infusion pump. Fig. 1 shows that a plateau for tyrosine is reached in the plasma which allows rates of protein synthesis to be calculated in a similar manner to that used for measuring muscle protein synthesis using the constant infusion method. This method has the advantage of procedural simplicity and also it expands the free amino acid pool so that error due to lack of precision in defining the precursor pool is reduced (Henshaw, Hirsch, Morton & Hiatt, 1971). One possible disadvantage of high levels of an amino acid in the precursor pool is that this amino acid at such levels may interfere with the regulation of protein synthesis. However, high levels of phenylalanine do not appear to affect the rates of protein synthesis in fish muscle as demonstrated by the leucine incorporation experiment (Table 1).

Rates of protein synthesis were calculated using the equations of Garlick *et al.* (1973). This equation (see Results) was derived for tissues with a low turnover rate such as muscle. It is likely that the time course for rise to plateau of tyrosine in the plasma and consequently within the muscle free amino acid pool will be different at different temperatures. However, when one examines the equation, it will be seen that there is no function present for the time course. This is because calculated values for fractional synthesis rates are very little affected even by large changes in the time course. In fact, it is not even necessary for a plateau level to be reached either in the plasma or the tissue during the course of the experiment for this equation to give accurate values for fractional rates of synthesis. Further detailed discussion of this subject and the assumptions inherent in the use of this equation can be found in a review by Waterlow, Garlick & Millward (1978).

The data presented in Fig. 2 show protein synthesis rates in the white muscle 24 h and 1 month after the animals had reached their new environmental temperature. The data at 24 h can be considered as the effect of temperature change *per se* upon protein synthesis as it is unlikely that any significant degree of compensation to the new thermal regime by metabolic reorganization could occur over such a short time period. The Q_{10} value of 3·57 obtained for white muscle protein synthesis rates in rainbow trout between 5 and 20 °C was higher than that obtained for carp of 3·07 between 10 and 25 °C, although both species had the same acclimation temperature of 15 °C. This discrepancy between the two species, however, appears to be an artifact of the different temperature ranges, as Q_{10} values between 10 and 20 °C in both species are not significantly different and approximate to 3·0. Thus, the discrepancy between the species when slightly different temperature ranges are used is due to highly reduced synthetic values at low temperatures. This is similar to the temperature response found in protein synthesis rates in the toadfish liver, where Q_{10} values were found to increase by a factor of 4 at low temperatures (Mathews & Haschemeyer, 1978). This also correlates with the Krogh metabolism *vs* temperature curve (Krogh, 1914). Only

one other study has looked at protein synthesis in skeletal muscle *in vivo* at different temperatures. Haschemeyer *et al.* (1979) measured protein synthesis in the white muscle of the brown triggerfish at 20 °C and 30 °C and obtained a Q_{10} value of just over 3, which compares well with the results obtained in this study for carp and trout.

Perhaps the most important finding of this study was that in the carp the rate of protein synthesis was modified as a result of temperature acclimation. Protein synthesis provides for replacement of large numbers of enzyme molecules which turn over rapidly and hence it constitutes a significant part of maintenance metabolism (Garlick, Burk & Swick, 1976). Studies in a variety of mammals have indicated that protein synthesis bears a constant ratio to local metabolic rate (Garlick *et al.* 1976; Nicholas, Lobley & Harris, 1977). Recent results in the liver of the toadfish have also suggested a correlation between the temperature dependency for protein synthesis and that of overall metabolism in poikilotherms (Haschemeyer *et al.* 1979). The elevation in synthesis rates with time in carp exposed to low temperatures corresponds to previously observed elevated enzyme levels and increased muscle oxidative capacity (Sidell, 1980) and remodelling of its myofibrillar system (Johnston, Davison & Goldspink, 1975; Penney & Goldspink, 1981; Johnston, 1979). Unless food is limited growth probably accounts for a fairly constant proportion of protein synthesis (Smith & Thorpe, 1976; Loughna & Goldspink, 1984) and hence elevated protein synthesis levels reduce the effect of temperature upon growth. In eurythermal species exposed to high environmental temperatures, adjustments in the rate of protein synthesis are also presumably necessary to reduce energy expenditure. Without an acclimatory effect, the increased synthesis rate (and hence metabolic rate) might outstrip the availability of resources such as food and oxygen.

This work shows that poikilothermic animals such as the carp have been able to evolve methods of modifying their rate of protein synthesis at different temperatures. The mechanism of the acclimatory changes in protein metabolism is being investigated further.

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