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Interaction of photosynthesis and respiration in Rhodospirillaceae: evidence for two functionally distinct b-c_1 complex fractions

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Flash-induced inhibition (on odd flashes) or reactivation (on even flashes) of respiration in photosynthetic bacteria has been interpreted formerly as resulting from a diversion of electrons from the respiratory pathway to the photosynthetic electron flow via cytochrome c_2 and re-injection of electrons in the respiratory pathway from the Q_A Q_B photoreduced acceptor (Verméglio A. and Carrier, J.M. (1984) Biochim. Biophys. Acta 764, 233–238); the model implied that the photooxidation of cytochrome c_2 was strictly concomitant with the rise of respiration inhibition and that this inhibition was of large magnitude. These consequences are not verified experimentally. Instead, when correctly interpreting the oxygen signal kinetics obtained with a new electrode system bringing by centrifugation a thin layer of cells in close contact with a platinum electrode, it is found that the respiration inhibition exhibits kinetics very similar to those of the re-reduction of cytochromes $c_1 + c_2$; also, they are always of small magnitude. A new model is proposed accounting for the above results: the respiratory and photosynthetic electron pathways are linked through the agency of the ubiquinone pool, not cytochrome c_2. It is further specified that two populations of b-c_1 complex coexist, one associated with the reaction center and the other with the cytochrome oxidase; these populations do not exchange freely, at least on a time-scale of a few seconds.

Introduction

The purple non-sulfur photosynthetic bacteria (Rhodospirilaceae) are capable of growing either phototrophically or aerobically in the dark [1]. When grown anaerobically in the light, they develop both photosynthetic and respiratory electron transfer chains located on the same continuous cytoplasmic membrane [2].

It has been known for many years that interactions occur between these two systems. A clear-cut piece of evidence of interaction is the total reversible inhibition of respiration observed under continuous illumination [3,4]. Two mechanisms underlie this phenomenon. First, the thermodynamic control exerted by the photosynthetic membrane potential at the level of the first two coupling sites of the respiratory chains (dehydrogenases) as previously proposed by McCarthy and Ferguson [5] and Cotton et al. [6]. This is prevalent under continuous illumination. A second type of mechanism involves changes in redox state of electron carriers common to both respiratory and photosynthetic chains [7,8]. We have provided strong evidence for this direct interaction between the electron transport chains under flashing light, using a fast amperometric method [9,10]. Only the cytochrome oxidase activity is affected under such excitation with short saturating flashes at low frequency [10]. Each odd flash induces an inhibition of the cytochrome oxidase activity, while respiration is restored after even flashes. We have therefore proposed that the flash-induced inhibition of respiratory activity was due to the diversion of electrons from the respiratory chain towards the photosynthetic reaction center via cytochrome c_2 [9,11], the oscillatory pattern being due to the re-injection of two electrons in the respiratory chain after even flashes as a consequence of the gating mechanism at the level of the secondary acceptor, Q_B [12,13]. This proposal is in agreement with the

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Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; c_1, cytochrome c_1 + cytochrome c_2; Q_A Q_B, quinone two-electron acceptor of reaction center; *Rps., Rhodopseudomonas; Rb., Rhodobacter.*

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results of Baccarini-Melandri et al. [14], who have shown by immunological studies that the soluble cytochrome c_2 is a direct electron donor to the cytochrome oxidase. In the present paper, we have correlated the kinetics, under short actinic saturating flashes, of both the fast changes of respiratory activity with a new O_2 monitoring electrode, and the photooxidation of reaction center electron donors by absorption change measurements. These experiments have been carried out with whole cells of two species of photosynthetic bacteria, *Rhodopseudomonas palustris* strain 2.1.6 and, to a lesser extent, *Rhodobacter sphaeroides* mutant strain Ga.

Material and Methods

Rps. palustris strain 2.1.6 and *Rb. sphaeroides* mutant stain Ga were grown in the light in degassed Hutner medium at 30 °C. The cells were harvested after 24 h of growth.

Absorption changes were performed with an apparatus similar to that in Ref. 15. To reduce sedimentation (and in part light-scattering), the bacteria were suspended in fresh growth medium containing 7% (w/v) Ficoll. Air was gently bubbled in the suspension to ensure aerobic conditions. Excitation was provided by a xenon lamp (flash duration: 2 μs). In order to improve the amperometric oxygen detection, we have developed a special type of electrode. It is a small cylindrical bucket of a capacity of about 3 ml. The bottom of the inside space is a flat circular platinum surface of about 2 cm^2 and the cylindrical wall is a silver surface (Ag/AgCl reference). The body of the device is made of epoxy resin; the two electrodes are actually partly included in this resin. The dimensions are such as to allow this device to fit snugly into the centrifuge tube of a swinging bucket rotor (SW 28, Beckman L8-55M ultracentrifuge). The bacterial cells, in a volume of 1–2 ml, are deposited in close contact with the platinum surface by spinning the electrode bucket at 15000 rpm ($30000 \times g$). This ensures a minimal mean diffusion path for oxygen between the active centres of the cell layer and the electrode, thereby increasing notably the sensitivity and speed of response compared to a conventional electrode system. The electrode circuit essentially consists of a low impedance current to voltage transformer; all operations in the amperometric measurement (including flash timing, A/D conversion and graphic display) are under the control of a microcomputer (Goupil 3, SMT). The operation time (from installation of the sample to actual O_2 measurement) can be as short as 15–20 mn.

Calculations on the kinetic model to be introduced below were performed using the Runge-Kutta algorithm for the numerical solution of sets of differential equations; a dedicated program was run on a G-40 (SMT) microcomputer.

Results and Discussion

In order to compare the kinetics of flash-induced inhibition/activation of the respiratory activity with that of cytochrome oxidation, we have performed amperometric measurements with the improved oxygen electrode, described above in the Material and Methods section. A successful comparison between both phenomena, amperometric response and light absorption changes, requires that the bacterial cells are in the same physiological conditions, in particular as regards the partial O_2 pressure.

One difficulty is found in the electrode measurements; it is related to an electrochemically induced anoxia at the level of the platinum electrode. The problem is best discussed by considering the three O_2 fluxes prevailing within the cell layer in contact with the electrode: the electrode consumption, the respiratory intake and the diffusive input. All three fluxes adjust mutually; however, the general trend is towards a decrease of all fluxes, due to the spatial extension of the O_2 gradient outwards away from the electrolyte/electrode interface. A state of variable anoxia may result from the competition between respiration and the electrode O_2 consumption. It is more severe the thicker the cell layer and/or the more negative the electrode polarization. Evidently, the flash-induced respiratory response disappears when the bacterial cells are in complete anoxia. Even under optimal conditions, anoxia will ultimately develop after prolonged electrode polarization, as the O_2 gradient continuously decreases and, with it, the diffusive O_2 flux. In the following experiments, we have in general used the least possible amount of bacterial cells, i.e., giving about a single cell layer on the platinum electrode after centrifugation, and kept the polarization low (−0.6 V), during as short a time as possible, in order to minimize anoxia.

A typical O_2 electrode experiment is seen in Fig. 1A for a pair of flashes spaced 1 s apart given to *Rps. palustris* whole cells. As already reported [9,10], a remarkable periodicity of two as a function of flash number is observed: on odd flashes, an inhibition of respiratory activity is induced (resulting in an increase of O_2 flux at the electrode, hence an increased signal), while on the contrary it is stimulated after even flashes (with the opposite effect on the electrode). The rather fast decrease of the amperometric signal observed after even flashes (second flash in Fig. 1A) makes it difficult to estimate the true half-time of the inhibition rise in this case. We have therefore restricted our attention to the kinetics of the inhibition rise induced by the first flash. Accordingly, Fig. 2 shows the flash-induced amperometric response (Fig. 2A) and absorption changes associated with cytochrome c_t ($c_2 + c_1$) oxidoreduction (Fig. 2B) observed in aerobic, dark-adapted *Rps. palustris* whole cells. The cytochrome

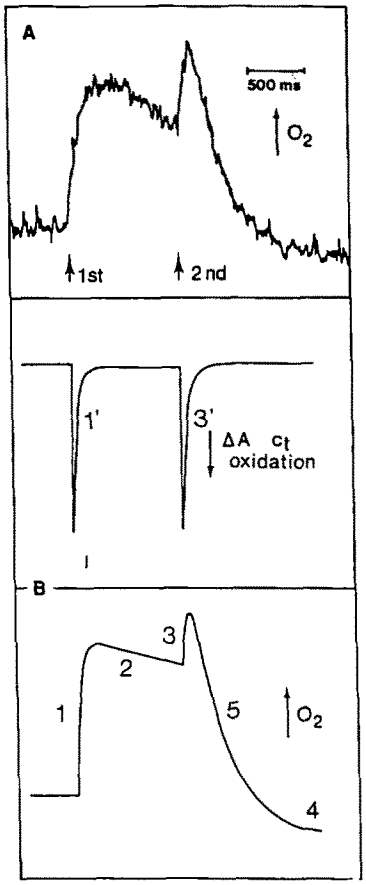

Fig. 1. (A) Flash-induced respiration changes in *Rps. palustris* measured with the O_2-centrifuged electrode. Cells suspended in KCl 0.1 M to give on the platinum surface after centrifugation a layer of optical absorbance 0.75 at 870 nm. Polarization: −0.6 V vs. Ag/AgCl. Temperature: 21° C. Two saturating flashes 1 s apart. (B) Simulation of flash-induced cytochrome c_t kinetics (top, downwards is oxidized) and respiration changes (bottom, upwards is inhibition). See scheme of Fig. 4B, where $k_1 = 0.025\ s^{-1}$; $k_2 = 0.2\ s^{-1}$; $k_3 = 0.1875\ U^{-1} \cdot s^{-1}$; $k_4 = 12.5\ U^{-1} \cdot s^{-1}$; $k'_4 = 0.0625\ u^{-1} \cdot s^{-1}$; $UQ_{total} = 20$ U; $c_{2,total} = 1$ u; $P_{total} = 1$ U. (U: arbitrary unit). Flash period = 1 s. The simulation was intended to give only a qualitative similarity with experimental curves (such as (A)), not an optimal fit.

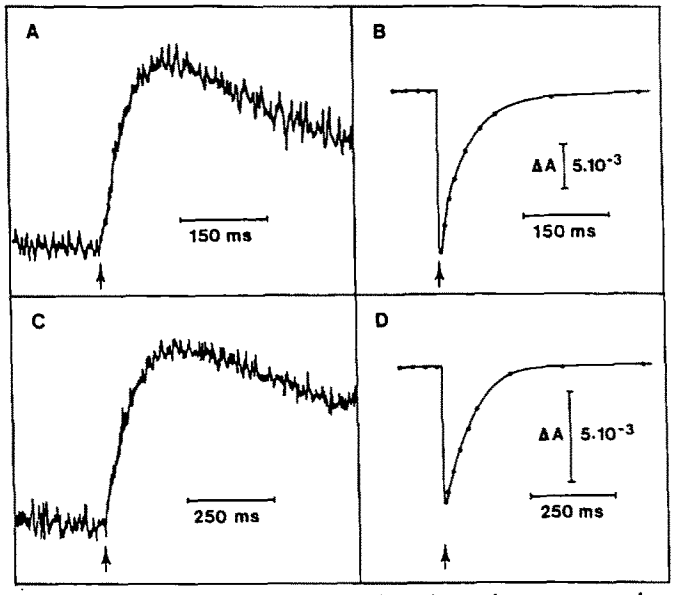

Fig. 2. Respiratory inhibition rise (A, C) and cytochrome c_t re-reduction (B, D) induced by a saturating flash on dark-adapted *Rps. palustris*. O_2 centrifuged electrode: conditions as in Fig. 1A; A: control; C: CCCP 1 μM, amplitude: 97% of control; note the change of time unit. Flash spectroscopy: light-induced absorption changes were measured at 422–435 nm; B: control; D: CCCP 1 μM; optical absorbance 1.0 at 870 nm.

photooxidation was observed at 422–435 nm, because of the much smaller contribution of the carotenoid band shift in this region as compared to the α band wavelength range. The c_t photooxidation is completed in less than 3 ms, in agreement with previous results [16–18]. The re-reduction in the dark does not clearly oscillate as a function of flash number (half-time: 30 and 25 ms for first and second flash, respectively). It is worth mentioning here that the aerobic condition, and hence the physiological state of the bacterial cells, in these series of experiments is quite different from the reducing condition generally used for ensuring an optimal cyclic photosynthetic electron transfer. In the presence of CCCP 1 μM, both kinetics are slowed down by a factor of about 2 (Fig. 2C and D), the amount of photooxidized c_t being decreased by about 50%. This is owing to a shift towards oxidation in the dark of the UQ pool and of cytochromes (data not shown) because of a faster respiratory activity due to CCCP uncoupling and limitation in rate of substrate oxidation in this condition [6]. Thus, there is a good correlation between the half times of re-reduction of cytochrome c_t and of the inhibition of respiration. In a series of independent experiments, the half-times of both phenomena have been compared (Fig. 3) and found close to equality; besides, their variations – be it under the effect of CCCP or due to the age of the culture – were well correlated. Similar observations were made using *Rb.*

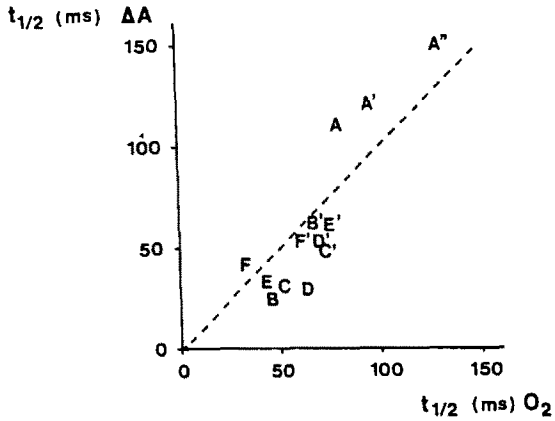

Fig. 3. Comparison of half-times of cytochrome c_t re-reduction and respiratory inhibition rise on first flash given to *Rps. palustris*. See Fig. 2. Capital symbols stand for independent bacterial samples (cultures were 1 day old, except for A which was 2 days); primed symbols: in the presence of CCCP (B', C', D', E': 0.5 μM; A', F': 1 μM; A": 2μM).

sphaeroides Ga (not shown), although the correlation was often only qualitative due to a tendency toward rapid inactivation of these cells during the O_2 measurements.

These results are in complete disagreement with the previous working hypothesis [11], where the oxygen signal rise on odd flashes is interpreted as a transient respiration inhibition due to the diversion of electrons out of the respiration pathway by the cytochrome c_2 photooxidation. Accordingly, the kinetics of the oxygen signal rise and cytochrome c_2 oxidation should be very similar and the restoration of the respiratory activity should follow the cytochrome re-reduction.

Concerning the O_2 signal rise, a trivial explanation could be that the speed of the amperometric response is strongly limited because of diffusion. A comparison with the response time in other similar photochemical systems (microalgal cells, chloroplasts) suggests that this is not the case. For instance, the half-time of the signal rise for the O_2 emission in *Chlorella* is about 5 ms. A more detailed argument is as follows. The electrode response has been simulated under the assumption of a strictly diffusion-limited process. An expression is derived for the electrode kinetics in response to a step of respiration change in an active layer at a distance, x, of the electrode in a medium where the diffusion coefficient for oxygen is D. In such a situation, the half-time of the respiration inhibition rise is found equal to 1.09 $x^2 \cdot D^{-1}$ (see Appendix). We did not know the parameter x and D for a layer of bacterial cells. For x, a rough estimate is to take half the diameter of a bacterial rod, i.e., about 0.5 μM. A lower limit is found assuming D is the same as in water ($3 \cdot 10^{-5}$ cm $\cdot$ s^{-1}); then the above inhibition half-time is equal to 90 μs. We have attempted to obtain an estimate of D under our working conditions by varying the thickness of the medium, x (Lavorel, unpublished data): this was obtained by layering a constant amount of (active) cells on top of a variable thickness of inactivated material. For technical reasons, the experiment was done only with *Chlorella* cells and isolated chloroplasts (O_2 evolution inactivated by mild heating). We obtain an estimate of $D = (2 \pm 1) \cdot 10^{-6}$ cm$^2 \cdot$ s^{-1}. If this D estimate is also correct for a bacterial cell, then the half-time of the respiration inhibition rise – if diffusion-limited – should be 1.4 ± 0.7 ms. Therefore, since the observed half-time is much larger than the above figure, it is reasonable to believe that the rise of the oxygen signal is not diffusion-limited, but rather kinetically limited, and that the good correlation observed between the half-times of re-reduction of cytochrome c_t and the inhibition of the respiratory activity is significant.

Aside from the above discrepancy, there was another incentive for re-examining the model. According to several lines of evidence, cytochrome c_2 is an integral carrier in the bacterial respiratory chain. Now, as c_t ($c_2 + c_1$) is about half photooxidized by a saturating flash, one should expect as a consequence a large transient inhibition of the respiratory activity, especially because cytochrome oxidase is present in very small amount compared to reaction centers [19]. Actually, we find that the flash-induced photoinhibition of respiration (e.g., 15% in *Rps. palustris*, 5% in *Rb. sphaeroides* Ga – not shown) is consistently much smaller than the photoinhibition of respiration in continuous light (100% or nearly so).

In order to take into account these discrepancies with the former model, i.e., the correlation of the respiration inhibition phase with c_t re-reduction and the small size of the inhibition amplitude, we propose a new model illustrated by the scheme of Fig. 4A. It is immediately apparent that the crossroads between the photosynthetic and the respiratory electron flow paths is no longer c_2 but is situated upstream in the UQ pool itself. Fig. 4B – a simplified presentation of the model (to be used below for the purpose of simulation) – clearly expresses this idea. A key feature in the new model is the assumption that the b-c_1 complexes and their associated c_2 are present in two functionally distinct fractions. A major fraction is located in the vicinity of the reaction centers where it functions normally as a member of the photosynthetic electron flow, while a minor fraction is located apart and connected to the cytochrome-oxidase. The inequality of the fractions is in agreement with literature data [19] and our comparison of respiratory and photosynthetic activities resulting in the stoichiometric ratios: center/c_2/b-c_1/cytochrome-oxidase being approximately $1/0.5/0.5/ < 0.01$. The two forms of b-c_1, although possibly distant on the membrane, are not isolated functionally. We assume that they both freely exchange electrons with a common UQ pool and that the electron transfers within the pool are not diffusion-limited, as appears to be the case for the plastoquinone pool of higher plants [20].

The association of the respiratory inhibition rise with the c_t re-reduction phase is explained as follows. Upon excitation with a saturating flash, the fraction $c_2 + b$-c_1

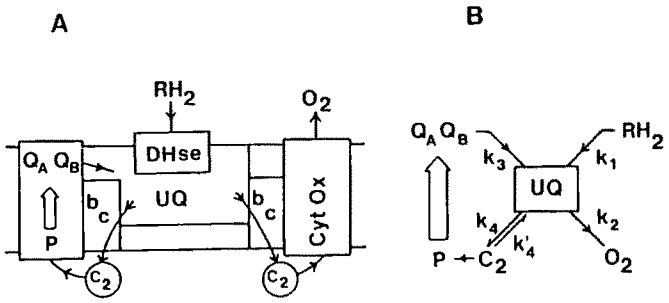

Fig. 4. Model of the interaction of photosynthetic and respiratory electron flows in Rhodospirillaceae (see text). (A) detailed topological version; (B) equivalent simplified version used in the numerical simulation.

associated with the reaction centres is quickly photooxidized; this is followed by its re-reduction by the reduced UQ in the pool. Because we assume a fast electron migration within the pool and a fast equilibration between UQ and c_t, the resulting electron deficit is immediately felt by the fraction of b-c_1 linked to cytochrome-oxidase, thus momentarily depressing the respiratory electron flux through cytochrome-oxidase. Since this fraction of c_t is small compared to that associated with the reaction center, its redox changes are not sensed practically by spectroscopy (at any rate, the changes are of same shape and opposite signs).

The small magnitude of the flash-induced respiratory inhibition is a consequence of the large redox span between UQ and c_t (about 100 mV, see Refs. 21, 22) and of the UQ pool being largely oxidized, the equilibrium being strongly shifted towards cytochrome c_t reduction. Due to the large equilibrium constant between c_t and UQ, the flash-induced electron deficit in UQ will therefore be translated into a comparatively smaller c_1 oxidation, unable to induce a complete inhibition of respiration.

We have simulated the amperometric and absorbance change responses according to the general scheme of Fig. 4B. Notice that the dark-adapted flux of electrons from the substrate RH_2 to oxygen through the quinone pool is simply accounted for here by two first-order rate constants: k_1 for input, k_2 for output; this, in particular, implies that the concentrations of RH_2 and oxygen are invariable. No detailed enzymatic mechanism is given; in particular, the role of the b-c_1 complex as a carrier linking c_2 to the UQ pool is not explicitly identified. In line with accepted ideas, it is assumed that the oxidation of reduced cytochrome c_t by the photooxidized primary donor, P, is instantaneous (compared to other relevant rates in the scheme) and that $Q_A\,Q_B$ transfers electrons only in pairs – i.e., when doubly reduced – to the quinone pool; as above stated, it is assumed that the UQ pool is strongly (not completely) oxidized, while, on the contrary, c_t is largely reduced. Fig. 1B shows the result of a simulation of cytochrome c_t absorption changes (top) and the O_2 electrode signal (bottom) induced by two flashes after dark adaptation. Concerning the latter signal, it is understood that the respiration rate is given by $k_2 \cdot (UQ)_{red}$ and it is further assumed that the electrode response is essentially proportional to the respiration rate – a correct assumption if diffusion is not at any time limiting. As was shown above, it is seen in this model that the rise of the respiration inhibition (phases 1 and 3) is concomitant with the cytochrome c_t re-reduction (phases 1′ and 3′) and that respiration subsequently slowly relaxes to its dark-adapted value (phases 2 and 4). As already stated, the binary oscillation of the O_2 signal results directly from the gating mechanism of the $Q_A\,Q_B$ reoxidation (phase 5).

Conclusion

Using a sensitive amperometric method, we have shown that the flash-induced inhibition of respiration is concomitant with the cytochrome c_t re-reduction. This behaviour is best understood and simulated using a kinetic scheme in which respiratory and photosynthetic electron transport chains are only connected via the UQ pool. The cytochrome c_2 and b-c_1 complexes, although integral carriers of both respiratory and photosynthetic chains, do not exchange freely between these two electron-transport systems, at least on a short time-scale. Our conclusions, although based on functional evidence, are consistent with the notion of stable supercomplexes involving the reaction center, the b-c_1 complex and cytochrome c_2 (Refs. 24, 25; Joliot, Joliot and Vermeglio, unpublished data); they are in agreement with former biochemical studies which have shown that the respiratory and photosynthetic electron transport chains are spatially separated in different areas of the intra-cytoplasmic membrane [23].

Appendix

Instead of the active layer experiencing a (negative) step of respiration change (problem A), let us consider the following related situation (problem B). The geometry is the same (active layer of negligible thickness at distance, x, from the electrode, diffusion coefficient, D, for oxygen), together with some usual, implicit assumptions (O_2 concentration zero at electrode plane, semi-infinit medium). Now, let us assume that at time $t = 0$ the active layer is instantaneously emitting a given quantity of oxygen. As it turns out, problem B is easier to solve than problem A and, given the solution of problem B, its time-integral is the solution of problem A.

The solution of problem B is (Lavorel unpublished data):

$$f(t) = \frac{x}{2\sqrt{(\pi D t^3)}} \exp(-x^2/4Dt) \qquad \text{(A1)}$$

On the time axis, $f(t)$ is pulse-shaped, with a sharp rising edge and a slower decay; its maximum is found at:

$$t_m = x^2/6D \qquad \text{(A2)}$$

The solution of problem A is therefore, to a constant factor:

$$F(t) = \int_0^t f(u)\,\mathrm{d}u \qquad \text{(A3)}$$

a monotonous, rising function of time, which can be obtained only numerically.

By comparing the graphical representations of $f(t)$ and $F(t)$, the half-rise time of $F(t)$, $t_{1/2}$, can be quantitatively compared to t_m, the time for the maximum of $f(t)$ and therefore, through Eqn. 2, $t_{1/2}$ can be expressed similarly as a function of x and D:

$$t_{1/2} = 1.09\, x^2/D \qquad \text{(A4)}$$

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