How to assay light responses in *Neurospora*

Luis M. Corrochano

Background

Blue light has several effects on the biology of *Neurospora*, including the activation of genes and biosynthetic pathways, and the induction of different developmental structures. The most conspicuous light response in *Neurospora* is the activation of the biosynthesis of the carotene neurosporaxanthin that results in deep-orange mycelia, but exposure to blue light can also lead to an increase in the amount of conida, protopheritecia, and a number of other developmental and physiological responses. The biology of the *Neurospora* responses to blue light has been reviewed by Linden et al. (1997).

How to assay the photoinduction of carotene biosynthesis

The mycelia of wild type *Neurospora* grown in the dark is white, but it turns deep orange soon after light exposure due to the activation of the genes for the enzymes required for carotene biosynthesis (Linden et al. 1997). Interestingly, the activation by light of the pathway for carotene biosynthesis is more effective when the cultures are exposed to light at low temperature (Harding et al. 1969). We grow the mycelia in the dark in Petri plates with liquid medium, and assay the photoinduction of carotene biosynthesis by exposing the plates to light at 8ºC (Arrach et al. 2002, Youssar et al. 2004). Light treatments at other low temperatures should work as well: we are currently exposing to light at 12ºC with results similar to those obtained at 8ºC. High temperatures (30-34ºC) can also be used for photoinduction but the mycelia accumulate a mixture of neurosporaxanthin and other carotenoids.

Procedure

Grow the desired number of plates containing each about 10^5-10^6 viable conidia in 25 ml of liquid Vogel's minimal medium with 0.2% Tween 80 as wetting agent. The plates are grown in the dark for two days (30ºC) and are then exposed to light at 8ºC for one day. After light exposure the mycelia are collected with the help of a tweezer, dried on filter paper, wrapped in aluminum foil, frozen in liquid nitrogen, and lyophilized.

Carotenoids are extracted from the samples by adding about 0.1 g of dried mycelia to 1 ml of acetone in 2 ml screw-cap tubes with 0.5 g of zirconium or glass beads. The mycelia are disrupted in a Mini beadbeater (Biospec) by a 30 sec-pulse followed by an incubation in ice. Then the tube is centrifuged, and the acetone phase is transferred to a rotoevaporation tube. The number of pulses depends on the amount of carotene in the sample. This procedured is repeated several times (typically 3-5 pulses) until the resulting mycelia is white and all the carotenoids have been extracted by acetone, and transferred to the rotoevaporation tube. An alternative procedure is to disrupt the dried mycelia with a mortar and pestle, and 1 g of sand, followed by acetone washing, and transfer to the rotoevaporation tube. The acetone sample is dried using the rotoevaporation tube, and dissolved in hexane as required. The hexane solution should allow a maximum optical density lower than 1. Light exposure at low temperature results in neurosporaxanthin as the major carotenoid, and allows an estimate of the total amount by measuring the maximal absorption in hexane (around 475-480 nm), and assuming an average E (1 mg/l, 1 cm)=200. For

photoinduction at high temperatures the amount of carotenoids can be estimated measuring the maximum absorption (around 475-480 nm), and assuming an average E (1 mg/l, 1 cm)=250.

The relative photoinduction can be obtained without the need to measure the absolute amount of neurosporaxanthin accumulated in the mycelia. The ratio of the maximum absorption between dark and light samples could be a good estimate of the effect of light on carotene biosynthesis, but the measurements of the absolute amounts of carotenes in each sample should always be preferred.

How to assay the photoinduction of gene expression

We routinely grow mycelia in the dark using standard Petri plates with liquid media. The plates are then exposed to light, and the mycelia harvested, and stored at -80ºC. Other alternatives are the use of 250-ml Erlenmeyer flasks with vigorous shaking for growing *Neurospora* in the dark. The resulting mycelia is then harvested in darkness with the help of a vacuum pump, and the mycelial pads are exposed to light (Corrochano et al. 1995). An easier alternative is to expose mycelia to light in 250-ml Erlenmeyer flasks (Shrode et al. 2001). The growth of *Neurospora* in Petri plates facilitates the handling of a large number of cultures and do not require the use of temperature-controlled shakers.

Procedure

Grow the desired number of plates containing each about 10^6 viable conidia in 25 ml of liquid Vogel's minimal medium with 0.2% Tween 80 as wetting agent. The plates are grown in the dark for one day (34ºC) or two days (22ºC) inside a dark box and then exposed to light. After light exposure the mycelia are collected with the help of a tweezer, dried on filter paper, wrapped in aluminium foil, frozen in liquid nitrogen, and stored at -80ºC. All the manipulations in the dark are done under red light. To ensure the safety of the red light, and to prevent blue-light contamination we routinely use a lamp with three red plexiglass filters.

The resulting mycelia are now ready for RNA or protein isolation. For RNA isolation we routinely break the mycelia with the help of a Mini beadbeater (Biospec) and use the Perfect RNA Eukaryotic mini kit (Eppendorf 955160045), but other procedures for RNA isolation should work as well.

How to assay the effect of light on hyphal branching

Neurospora cultures grown in the light show higher branching than cultures kept in the dark. As a result, colonies grown in the light are more compact than those grown in the dark (Lauter et al. 1998; Ambra et al. 2004).

Procedure

The observation of hyphal growth can be performed using different procedures. The simplest procedure is to inoculate one or several colonies in a sorbose agar plate to prevent aerial growth. The plates are grown at 34 ºC for one day in the dark or under light exposure, and the edge of each growing colony is photographed with an inverted microscope. An alternative procedure is to apply a sample of 10^3 conidia in Vogel's medium to an sterile microscope depression slide, the sample is covered with cover slips, and incubated in moist chambers (Lauter et al. 1998).

How to assay the photoinduction of protopheritecia development
The female sexual structures (protoperithecia) of *Neurospora* are induced by blue-light illumination (Degli-Innocenti et al. 1983). The effect of light on protoperithecia formation is enhanced if the culture medium lacks nitrogen. Under these conditions, the formation of conidia is strongly reduced.

Procedure
Plates of Westergaard medium with a modified trace elements composition (5.25 µg $Na_2B_4O_7$ 10 H_2O, 100 µg $CuCl_2$ 2 H_2O, 200 µg $FeCl_3$ 6 H_2O, 20 µg $MnCl_2$ 4 H_2O, 20 µg $(NH_4)_6$ 6 Mo_7O_{24} 4 H_2O, 2 µg $CoCl_2$) (Degli-Innocenti et al. 1983) are inoculated each with 10^4 conidia. The mycelia are grown in the dark at 26ºC for three days, then the plates are exposed to light, and incubated in the dark for one more day before counting the protoperithecia under a dissecting microscope. A light exposure of the appropriate intensity for one minute should be enough to promote perithecial development. A detailed fluence/response curve is described in Degli-Innocenti et al. (1983). Other media composition and incubation temperatures should also allow the visualization of this light effect, but the duration of the incubations in the dark before and after light exposure should be optimized.

How to assay phototropism and the effect of light on the polarity of the perithecial beak
The shape of fertilized perithecia culminates with a beak that is located at the top when the culture has been illuminated. However, cultures kept in the dark form beaks at a random location on each perithecium (Oda and Hasunuma 1997). In addition, the perithecial beaks show a positive phototropism when illuminated with blue light (Harding and Melles 1983).

Procedure
The effect of light on the polarity and the phototropism of the perithecial beak requires that we set up a cross using the standard procedures and media. After fertilization the cultures are kept at 25ºC in the dark for three days and then exposed to cycles of 12 hours light/12 hours dark during 14 days. The beaks are scored under a dissecting microscope. The effect of light on the polarity of the perithecial beak can be observed with cultures illuminated from the top of the plate. However, for the observation of phototropism it is necessary to set up an illumination chamber that provides light from the side of the Petri plate. When we prepare the cross, it is wise to add the male conidia to a line across the plate. A fertilization line at the diagonal of the Petri plate will provide a nice line of perithecia for the observation of phototropism (Harding and Melles 1983; Oda and Hasunuma 1997).

How to assay the effect of light on the circadian clock
Neurospora band strains grown in race tubes under continuous light produce conidia continuously. However, a clear banding pattern of clock-controlled conidiation is observed in the dark. It is therefore relatively simply to measure the intensity of light required for the light-suppression of clock-controlled conidiation. In addition, light exposure can advance the phase of the circadian

rhythm, and it is possible to measure this effect in a quantitative way (Crosthwaite et al. 1995).

Procedure
Race tubes are inoculated with conidia and incubated in constant light for two days. The race tubes are then transferred to darkness and incubated for a further 32.5 h in the dark before exposing them to brief pulses of light (10-120 sec). The tubes are then returned to the dark and the circadian cycles and phase changes scored after several days of growth (Crosthwaite et al. 1995).

How to assay the photoinduction of conidia development
Conidiation in *Neurospora* is a developmental process that is induced by several environmental cues such as desiccation, lack of nitrogen or carbon, and the presence of light (reviewed by Springer 1993). Light is required to obtain the maximum number of conidia, but it produces only a modest fourfold increment in the amount of conidia over that obtained in cultures kept in the dark (Lauter et al. 1997).

Procedure
Vogel's minimal agar slants are inoculated with about 10^4 conidia, and are incubated for 7 days at 25ºC in the dark or under constant light. The conidia produced by each culture are collected by adding 5 ml of sterile distilled water to each tube, and shaking the tube vigorously to release most conidia from the mycelia. The conidial suspension is tranferred to a tube and centrifuged. Then, the conidial pellet is resuspended in 1 ml of sterile distilled water, and the conidia in one sample (typically 10 µl) are counted with the help of a hemocytometer (Lauter et al. 1997). Other temperatures should also allow the measurement of the effect of light on conidial production, but the incubation time required to obtain a high conidial yield should be investigated.

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