Nitrogen regulation of blue light-inducible genes in *Neurospora crassa*

VLADIMIR Y. SOKOLOVSKY,¹† FRANK-ROMAN LAUTER,¹‡* BERND MÜLLER-RÖBER,¹§
MARTA RICCI,¹ THOMAS J. SCHMIDHAUSER² and VINCENZO E. A. RUSSO¹

¹Max-Planck-Institut für Molekulare Genetik, Abteilung Trautner, Ihnestr. 73, 1000 Berlin-Dahlem 33, Germany

²Department of Chemistry and Biochemistry, Southern Illinois University Carbondale, Carbondale, Illinois 62901, USA

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Sexual and asexual differentiation of *Neurospora crassa* are influenced by nitrogen availability and blue light. Nitrogen limitation induces the production of protoperithecia on solid medium and conidia in liquid medium. Both developmental processes are stimulated by blue light. We have analysed changes in mRNA levels for a variety of light-inducible genes (*al*, *bli* and *con*) under conditions of nitrogen limitation. We show that the photoregulated genes *al-1*, *al-2*, *bli-4*, *bli-7*, *con-5* and *con-10* are also regulated by nitrogen limitation. These genes exhibited nitrogen regulation in the nonphotoresponsive mutant strains *wc-1* and *wc-2*. Therefore, the *wc-1* and *wc-2* gene products, although necessary for photoregulation of these *al*, *bli* and *con* genes, are not required for nitrogen regulation.

Introduction

During its life cycle, the heterothallic filamentous fungus *Neurospora crassa* undergoes asexual and sexual differentiation. Both developmental processes are influenced by external signals, primarily nutrient deprivation and illumination with blue light (Nelson *et al.*, 1975; Turian, 1977; Degli-Innocenti & Russo, 1984*a, b*).

The vegetative growth of the fungus is characterized by the formation of mycelia composed of multinucleate, branched hyphae. In liquid shake cultures, nitrogen limitation activates the developmental process, resulting in production of conidia (Guignard *et al.*, 1984; Müller & Russo, 1989). These semi-dormant spores represent the final products of asexual differentiation. This process of conidiation involves a series of discrete morphological stages which have been characterized in detail by scanning electron microscopy (Springer & Yanofsky, 1989). On solid medium, conidiation is induced by desiccation (Nelson *et al.*, 1975) and glucose limitation (Ricci *et al.*, 1991). Once induced, several events in this complex differentiation process are influenced by light, e.g. conidiophores develop towards light (Faull, 1930; Siegel *et al.*, 1968), and illuminated cultures produce

conidia faster than dark-grown cultures (Siegel *et al.*, 1968; Turian, 1977).

On solid medium, nitrogen limitation leads to the differentiation of hyphae into the female organs, protoperithecia (Sommer *et al.*, 1987; Ricci *et al.*, 1991). During the sexual process these are fertilized by conidia or vegetative hyphae of the opposite mating type. Once induced by nitrogen deprivation, the formation of protoperithecia is greatly enhanced by blue light (Degli-Innocenti *et al.*, 1983; Sommer *et al.*, 1987). In dark-grown mycelia about 10 protoperithecia form per cm²; this increases to about 300 per cm² after blue light illumination (Sommer *et al.*, 1987).

Recently, two sets of genes have been identified which are of interest with respect to the developmental programs in *N. crassa*. One group, the *con* genes, was isolated on the basis of preferential expression during conidiation (Berlin & Yanofsky, 1985). The functions of these genes are unknown. The second group comprises blue-light-inducible (*bli*) genes; mRNAs corresponding to these genes increase after illumination with blue light (Sommer *et al.*, 1989). The functions of the *bli* genes, and their expression pattern during conidiation are also unknown. Interestingly, some of the *con* genes respond to both light and conidiation (Lauter & Russo, 1991). Two additional genes that are also both light-inducible and expressed during conidiation, albino-1 (*al-1*) (Schmidhauser *et al.*, 1990; T. J. Schmidhauser, M. S. Sachs & C. Yanofsky, unpublished data) and albino-2 (*al-2*) (T. J. Schmidhauser, F.-R. Lauter, V. E. A. Russo & C. Yanofsky, unpublished data), were used in this analysis.

* Author for correspondence. fax (415) 723 6132.

† Permanent address: A. N. Bach Institute of Biochemistry, 33 Leninsky Prospekt, Moscow 117071, USSR.

‡ Present address: Department of Biological Sciences, Stanford University, Stanford, California 94305-5020, USA.

§ Present address: Institut für Genbiologische Forschung, Ihnestr. 63, 1000 Berlin-Dahlem 33, Germany.

The *al* genes are necessary for carotenoid production (Harding & Turner, 1981). *al-1* encodes the enzyme phytoene dehydrogenase (Schmidhauser *et al.*, 1990) and *al-2*, phytoene synthase (T. J. Schmidhauser, F.-R. Lauter, V. E. A. Russo & C. Yanofsky, unpublished results) of *N. crassa*. Although they are developmentally regulated, expression of these genes is not necessary for asexual and sexual development (Degli-Innocenti & Russo, 1984a; Russo, 1986).

Two regulatory mutants of *N. crassa*, *wc-1* and *wc-2*, are impaired in light regulation. The products of *wc-1* and *wc-2* are required for all photoeffects tested so far (Harding & Turner, 1981; Harding & Melles, 1984; Degli-Innocenti & Russo, 1984a, b; Russo, 1988; Sommer *et al.*, 1989; Nelson *et al.*, 1989; Schmidhauser *et al.*, 1990; Nawrath & Russo, 1990; Lauter & Russo, 1991), with the exception of photophosphorylation of proteins (Lauter & Russo, 1990). *Wc-1* and *Wc-2* are thought to be regulatory factors responsible for the light transduction pathway.

We report here that the light-regulated genes *al-1*, *al-2*, *bli-4*, *bli-7*, *con-5* and *con-10* are also regulated by nitrogen limitation. Transcript accumulation of those genes is increased upon nitrogen limitation in wild-type as well as *wc-1* and *wc-2* mutant strains.

Methods

Strains and plasmids. *N. crassa* wild-type (WT) strain (St. Lawrence, STA) was provided by J. R. S. Fincham (Cambridge University, UK). Strains FGSC 4398 (*wc-1*, a), FGSC 4396 (*wc-1*, a), FGSC 4408 (*wc-2*, a) and R251 (*wc-2*, a) were used as sources of *wc* RNA. The *wc* strains were isolated after UV mutagenesis and are isogenic to the WT (STA) (Degli-Innocenti & Russo, 1984a). The *con* clones were provided by Charles Yanofsky (Stanford University) (Berlin & Yanofsky, 1985; Roberts *et al.*, 1988). The *al-1* and *bli* genes were described previously (Schmidhauser *et al.*, 1990; Sommer *et al.*, 1989). In case of *al-2*, an 800 bp cDNA fragment cloned in pTJS430 was used as hybridization probe.

Growth of *N. crassa* in liquid culture medium. *N. crassa* mycelia were grown in liquid culture, with shaking, as described by Chambers *et al.* (1985). Mycelia were grown in darkness in 75 ml medium in 250 ml flasks at 34 °C for 24 h prior to harvest. Modified Vogel's minimal medium (Russo, 1988) supplemented with 2% (w/v) glucose and 50 mM-NH₄Cl as carbon and nitrogen sources, respectively, was used for cultivation. In nitrogen limitation experiments, mycelia were grown in the dark for 24 h in a medium supplemented with 2 mM-NH₄Cl. Mycelia were harvested under red safety light by filtration (Chambers *et al.*, 1985) and immediately frozen in liquid nitrogen.

Culture of *N. crassa* on solid medium. *N. crassa* was grown in 8 cm diameter Petri dishes containing 20 ml agar medium. The agar medium was modified Vogel's minimal medium (Russo, 1988) supplemented with 1.5% (w/v) Bacto-Agar (Difco), with 1% (w/v) L-sorbose, and 0.1% glucose as carbon sources, with 4 mM-NH₄Cl as the sole nitrogen source. Solid medium was covered with a circular dialysis membrane (Visking, Serva). For inoculation, 10⁴ conidia per plate were spread onto the dialysis membrane. Mycelia were grown in darkness at 23 °C. After 3 d, membranes overgrown with mycelia were transferred onto

new plates that contained zero or 50 mM-NH₄Cl as sole nitrogen source. After 24 h additional growth in darkness at 23 °C, cells were harvested by scraping them from the upper surface of the dialysis membranes. All manipulations were performed under a red safety light. Collected mycelia were immediately frozen in liquid nitrogen.

RNA dot blot analysis. Extraction of total RNA was performed as described previously (Sokolovsky *et al.*, 1990), an extraction method developed for RNAase-rich cultures. RNA dot-blots were performed as described by Boll *et al.* (1986). Radioactively labelled DNA probes were prepared using random primer labelling technique (Feinberg & Vogelstein, 1983). Quantification of mRNA was done as described previously (Sommer *et al.*, 1989).

Results

Nitrogen regulation of light-inducible genes in mycelia grown in liquid and on solid medium

We wished to determine the expression patterns of light-regulated genes in response to nitrogen availability, a stimulus known to influence development. Nitrogen limitation induces conidiation in liquid media (Guignard *et al.*, 1984; Müller & Russo, 1989), and both inhibits conidiation (Ricci *et al.*, 1991) and induces protoperithecia formation (Sommer *et al.*, 1987; Ricci *et al.*, 1991) on solid media. Since different developmental processes may be induced by the same stimulus, depending on growth conditions, we have compared the effects of nitrogen limitation on the transcription patterns of photoregulated genes in mycelia grown in liquid culture and on solid agar. Cultures were grown in darkness under conditions of low or high nitrogen supply, and the amount of mRNA expressed from different genes was measured by dot-blot analysis (Boll *et al.*, 1986). Our results, based on RNA extracted from three to five different mycelial cultures grown in liquid medium and two different mycelial cultures grown on solid medium, are compared in Table 1.

Under conditions of nitrogen limitation, mRNA levels for all the photoregulated genes except *bli-3* increased by more than a factor of 3 in wild-type cultures grown in liquid medium. The slight increase in transcript levels of *bli-3* was not considered significant. A greater than 10-fold increase in accumulation was observed for the *al-1* and *bli-7* RNAs. The increase in mRNA level for *bli-7*, a gene which is weakly photoregulated (Sommer *et al.*, 1989), was appreciable; a greater than 50-fold accumulation was observed. In the induced state, the *bli-7* mRNA represented about 0.25% of the total RNA (Table 1). Under conditions of nitrogen limitation, the variation in *bli-7* transcript levels among different experiments was fairly low (less than 10%). However, fluctuation of *bli-7* transcript levels has been observed in mycelia grown in complete medium (Tables 1 and 2;

Table 1. Gene regulation in response to nitrogen limitation using liquid and solid growth media

Total RNA was isolated from dark-grown wild-type (STa) *N. crassa* cultures, with low (N−) or high (N+) nitrogen supply (see Methods). mRNA accumulation from seven photoinducible (top group) and two photoindependent (lower group) genes was monitored by dot-blot analysis. See Methods for mRNA quantification. Data are means from 3–5 independent RNA extractions ($\pm$ SEM) for liquid cultures; for solid cultures, two values (a, first experiment; b, second experiment) are quoted.

Gene	mRNA levels (pg/ μ g)			
	Liquid medium		Solid medium	
	N+	N−	N+	N−
			a/b	a/b
<i>al-1</i>	4.5 ($\pm$ 1.6)	63.7 ($\pm$ 5.8)	3.5/11	13/120
<i>al-2</i>	1.2 ($\pm$ 0.4)	8.6 ($\pm$ 2.0)	1.8/1.8	12/20
<i>bli-3</i>	11.4 ($\pm$ 3.1)	14.7 ($\pm$ 0.8)	14/29	15/43
<i>bli-4</i>	1.2 ($\pm$ 0.3)	4.1 ($\pm$ 0.7)	3/5	9/26
<i>bli-7</i>	52.1 ($\pm$ 21.7)	2699.2 ($\pm$ 256.2)	19/142	27/67
<i>con-5</i>	11.4 ($\pm$ 3.2)	47.2 ($\pm$ 4.5)	10/27	21/130
<i>con-10</i>	0.6 ($\pm$ 0.2)	2.7 ($\pm$ 0.8)	0.5/10	0.5/4
<i>am</i>	11.9 ($\pm$ 2.4)	190.0 ($\pm$ 16.7)	75/47	88/59
<i>tub-2</i>	3.7 ($\pm$ 0.4)	8.5 ($\pm$ 2.0)	22/19	44/41

Sommer *et al.*, 1989). Two nonphotoregulated genes; *tub-2*, which encodes β -tubulin, a subunit of cytoskeleton microtubules (Orbach *et al.*, 1986), and *am*, which encodes NADP-specific glutamate dehydrogenase (GDH) of *N. crassa* were included for comparison. GDH is an enzyme of the nitrogen assimilatory pathway and converts ammonia to glutamate (Kinnard *et al.*, 1982). Expression of *tub-2* was slightly increased under conditions of nitrogen limitation. Transcription of *am* was under nitrogen control in liquid medium (Table 1); an induction factor greater than 10 was observed.

In two independent experiments, nitrogen regulation was also analysed in mycelia grown on solid agar. As in liquid medium, transcript levels of *al-2* and *bli-4* increased under conditions of nitrogen limitation (Table 1). The induction factors were comparably high under both growth conditions. In case of *al-1*, *con-5* and *con-10*, the variation between the two experiments was too high to infer whether or not the mRNA levels of those genes increased under conditions of nitrogen limitation. As in liquid medium, transcript levels of *tub-2* increased slightly in cultures shifted to a nitrogen-free medium; the slight increase in mRNA levels of *bli-3* was not considered significant. Interestingly, transcription of *am* and *bli-7*, although strongly nitrogen-regulated in mycelia grown in liquid cultures, were uninducible by nitrogen limitation in mycelia grown on solid medium. This effect was especially significant in the case of *bli-7*. Under conditions of nitrogen limitation, *bli-7* mRNA, measured in cultures grown on solid agar medium,

represented only 1–2 % of the amount measured in cultures grown in liquid medium.

Role of *wc* genes on nitrogen induced gene expression

Nitrogen regulation of photoinducible genes was also examined in *wc* strains. The *wc* mutants are blind for most of the photoeffects tested so far. Transcripts of the photoregulated *al* (Schmidhauser *et al.*, 1990; Nelson *et al.*, 1989; F.-R. Lauter, T. J. Schmidhauser, C. Yanofsky & V. E. A. Russo, unpublished results), *bli* (Sommer *et al.*, 1989) and *con* genes (Lauter & Russo, 1991) do not increase after light stimulation in *wc-1* and *wc-2* strains.

In order to study nitrogen regulation in these mutants, the fungus was grown in darkness in liquid medium with low or high nitrogen supply. Two alleles each of *wc-1* and *wc-2* were used. Results are presented in Table 2. The response of *al-1*, *al-2*, *bli-3*, *bli-4*, *bli-7* and *tub-2* to nitrogen limitation was similar to WT in the *wc* mutant strains. RNA accumulation for *con-5*, *con-10* and *am* was slightly altered in *wc* strains following nitrogen limitation relative to wild-type, but still nitrogen-regulated. The products of the *wc-1* and *wc-2* genes do not appear to be required for nitrogen regulation of the genes analysed in these studies.

Discussion

Sexual as well as asexual differentiation of *N. crassa* is induced by nitrogen limitation (Nelson *et al.*, 1975; Guignard *et al.*, 1984; Sommer *et al.*, 1987; Müller & Russo, 1989; Ricci *et al.*, 1991). Both developmental processes are also influenced by light (Faull, 1930; Sargent *et al.*, 1966; Sargent & Briggs, 1967; Siegel *et al.*, 1968; Turian, 1977; Degli-Innocenti *et al.*, 1983; Sommer *et al.*, 1987). We have analysed transcript levels of blue-light-inducible genes under conditions of nitrogen limitation. In cultures grown in liquid medium the amounts of *al-1*, *al-2*, *bli-4*, *bli-7*, *con-5* and *con-10* mRNAs were also regulated by nitrogen availability in the medium. Therefore, these genes are under dual environmental control, being regulated by either nitrogen or light.

In addition, two non-photoregulated genes, *tub-2* and *am*, were analysed. *tub-2* RNA levels were independent of the nitrogen supply, but *am* transcripts increased by a factor of more than 10 when nitrogen was limiting. NADP-specific GDH activity (Dantzig *et al.*, 1979) and *am* mRNA levels are known to be under nitrogen control (Frederick & Kinsey, 1990).

The influence of a third environmental parameter, liquid versus solid growth media, was also analysed. In the laboratory, *N. crassa* is normally grown either in

Table 2. *Gene regulation under nitrogen limitation: wild-type vs. wc-1 and wc-2 mutant strains*

Total RNA was isolated from *N. crassa* cultures dark-grown in liquid medium with high or low nitrogen supply (see Methods). Accumulation of specified transcripts was monitored by dot-blot analysis and quantified by densitometry. Values are means ($\pm$ SEM) from 3–5 independent extractions per growth condition and strain; ratios were calculated for each high/low N pair and mean ratios then calculated. All data were normalized to *n-6* (Sommer *et al.*, 1989), a nitrogen-independent gene (not shown). Two alleles of each *wc* locus were used. R251 is a *wc-2* strain isolated in the Russo laboratory after UV mutagenesis, isogenic to the wild-type STa.

Gene	WT	Relative mRNA levels N–/N+			
		<i>wc-1</i> 4398	<i>wc-1</i> 4396	<i>wc-2</i> 4408	<i>wc-2</i> R251
<i>al-1</i>	20.8 ($\pm$ 5.8)	25.6 ($\pm$ 7.8)	35.1 ($\pm$ 7.4)	48.3 ($\pm$ 15.0)	20.0 ($\pm$ 9.5)
<i>al-2</i>	7.5 ($\pm$ 2.6)	5.1 ($\pm$ 0.8)	12.1 ($\pm$ 2.7)	9.3 ($\pm$ 3.9)	7.1 ($\pm$ 1.2)
<i>bli-3</i>	1.9 ($\pm$ 0.7)	1.9 ($\pm$ 0.8)	1.1 ($\pm$ 0.5)	1.8 ($\pm$ 0.7)	1.1 ($\pm$ 0.3)
<i>bli-4</i>	4.1 ($\pm$ 0.9)	2.5 ($\pm$ 0.5)	5.0 ($\pm$ 1.1)	3.7 ($\pm$ 0.8)	2.3 ($\pm$ 0.6)
<i>bli-7</i>	176.5 ($\pm$ 87.2)	80.4 ($\pm$ 23.4)	153.0 ($\pm$ 91.3)	85.0 ($\pm$ 19.1)	65.0 ($\pm$ 24.6)
<i>con-5</i>	4.1 ($\pm$ 0.4)	2.0 ($\pm$ 0.4)	3.0 ($\pm$ 0.2)	2.0 ($\pm$ 0.4)	1.8 ($\pm$ 0.6)
<i>con-10</i>	4.2 ($\pm$ 1.2)	8.7 ($\pm$ 1.3)	9.2 ($\pm$ 1.1)	7.7 ($\pm$ 1.1)	6.1 ($\pm$ 2.9)
<i>am</i>	17.8 ($\pm$ 4.0)	5.1 ($\pm$ 0.8)	20.1 ($\pm$ 7.3)	5.8 ($\pm$ 1.6)	7.3 ($\pm$ 2.6)
<i>tub-2</i>	2.1 ($\pm$ 0.6)	1.0 ($\pm$ 0.4)	1.9 ($\pm$ 0.5)	1.3 ($\pm$ 0.3)	1.3 ($\pm$ 0.2)

WT, wild-type.

shaking liquid medium, submerged, or on solid agar. In liquid medium, nitrogen and glucose starvation leads to conidiation (Guignard *et al.*, 1984; Müller & Russo, 1989), and no protoperithecia. On solid agar, nitrogen limitation induces protoperithecia formation (Sommer *et al.*, 1987) and suppresses conidiation, while glucose limitation induces conidiation and suppresses protoperithecia formation (Ricci *et al.*, 1991). In our experiments, despite their limited number and the variation between them, it was apparent that the expression of *bli-7* and *am* differed significantly in cultures grown in liquid and on solid medium.

RNA levels for *bli-7* are strongly nitrogen-regulated in liquid medium but unregulated on solid agar. This implies that *bli-7* is regulated by at least three parameters; light, nitrogen availability, and the method of cultivation (liquid versus solid medium). Gene inactivation experiments should show whether the changes in *bli-7* mRNA levels were accidentally parallel to the morphological transformations associated with conidiation or whether the product of this gene is essential for normal asexual differentiation. The amount of *bli-7* mRNA in cells grown in liquid medium under conditions of nitrogen limitation was 0.25% of the total RNA. Assuming that *bli-7* mRNA is polyadenylated, that poly (A⁺) mRNA represents 1–2 % of the total RNA of *N. crassa* (Sachs & Yanofsky, 1991) and that the relative amounts of rRNA and tRNA do not change during nitrogen starvation, the amount of *bli-7* mRNA can be estimated to be 10–25 % of the total poly (A⁺) mRNA mass.

Why is *bli-7* nitrogen-regulated in *N. crassa* grown in liquid but not on solid medium? One important

difference between liquid and solid medium is the amounts of O₂ and CO₂ that are available to the organism. It is known that CO₂ inhibits conidiation in *N. crassa* (Charles, 1962; Sargent & Kaldenborn, 1972) and differentiation of the fungus *Phycomyces* (Russo *et al.*, 1981).

How is gene regulation by light and nitrogen achieved in *N. crassa*? Which elements participate in these regulatory processes? Two genes, *wc-1* and *wc-2*, are likely to play an important role in the blue-light signal transduction chain of *N. crassa* (Harding & Turner, 1981; Degli-Innocenti & Russo, 1984b) and to influence proper dephosphorylation of proteins (Lauter & Russo, 1990). Their gene products are necessary for photocarotenogenesis (Harding & Turner, 1981), phototropism of perithecial beaks (Harding & Melles, 1984), photoinduction of protoperithecia (Degli-Innocenti & Russo, 1984a) and photoinduction of circadian rhythms in *bd* mutants (Russo, 1988). In mycelia of *wc-1* and *wc-2* mutant strains, transcription of *al*, *bli* and *con* genes (Sommer *et al.*, 1989; Nelson *et al.*, 1989; Schmidhauser *et al.*, 1990; Lauter & Russo, 1991) is not photoinducible.

The results presented here show that nitrogen regulation of *al-1*, *al-2*, *bli-4*, *bli-7*, *con-5* and *con-10* was not dramatically altered in *wc* mutant strains. Therefore, the products of *wc-1* and *wc-2*, while absolutely necessary for light regulation of *al*, *bli* and *con* genes, are not needed for their nitrogen regulation.

RNA accumulation of *al-1*, *al-2*, *bli-4*, *bli-7*, *con-5* and *con-10* is regulated by two environmental factors that also influence development of *N. crassa*: nitrogen availability and blue light. Gene inactivation experiments should show whether some of the *bli* or *con* genes

are needed for sexual or asexual differentiation processes in this fungus. Deletion analyses and study of protein-DNA interactions should lead to the identification of *cis* and *trans* acting elements that are involved in dual environmental control of those genes.

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References

- BERLIN, V. & YANOFSKY, C. (1985). Isolation and characterization of genes differentially expressed during conidiation of *Neurospora crassa*. *Molecular and Cellular Biology* **5**, 849-855.
- BOLL, W., FUJISAWA, J.-I., NIEMI, J. & WEISMANN, C. (1986). A new approach to high sensitivity differential hybridization. *Gene* **50**, 41-53.
- CHAMBERS, J. A. A., HINKELAMMERT, K. & RUSSO, V. E. A. (1985). Light-regulated protein and poly(A)⁺ mRNA synthesis in *Neurospora crassa*. *EMBO Journal* **4**, 3649-3653.
- CHARLES, H. P. (1962). Response of *Neurospora* mutants to carbon dioxide. *Nature, London* **195**, 359-360.
- DANTZIG, A. H., WIEGMANN, JR, F.-L. & NASON, A. (1979). Regulation of the glutamate dehydrogenase in *nit-2* and *am* mutants of *Neurospora crassa*. *Journal of Bacteriology* **137**, 1333-1339.
- DEGLI-INNOCENTI, F., POHL, U. & RUSSO, V. E. A. (1983). Photoinduction of protoperithecia in *Neurospora crassa* by blue light. *Photochemistry and Photobiology* **37**, 49-51.
- DEGLI-INNOCENTI, F. & RUSSO, V. E. A. (1984a). Isolation of new white collar mutants of *Neurospora crassa* and studies on their behavior in the blue-light-induced formation of protoperithecia. *Journal of Bacteriology* **159**, 757-761.
- DEGLI-INNOCENTI, F. & RUSSO, V. E. A. (1984b). Genetic analysis of blue-light-induced responses in *Neurospora crassa*. In *Blue Light Effects in Biological Systems*, pp. 214-219. Edited by H. Senger. Berlin: Springer Verlag.
- FAULL, A. F. (1930). On the resistance of *Neurospora crassa*. *Mycologia* **22**, 288-303.
- FEINBERG, A. P. & VOGELSTEIN, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry* **132**, 6-13.
- FREDERICK, G. D. & KINSEY, J. A. (1990). Distant upstream regulatory sequences control the level of expression of the *am* (GDH) locus of *Neurospora crassa*. *Current Genetics* **18**, 53-58.
- GUIGNARD, R., GRANGE, F. & TURIAN, G. (1984). Microcycle conidiation induced by partial nitrogen deprivation in *Neurospora crassa*. *Canadian Journal of Microbiology* **30**, 1210-1215.
- HARDING, R. W. & MELLES, S. (1984). Genetic analysis of the phototropism of *Neurospora crassa* perithecial beaks using white collar and albino mutants. *Plant Physiology* **72**, 996-1000.
- HARDING, R. W. & TURNER, R. V. (1981). Photoregulation of the carotenoid biosynthetic pathway in albino and white collar mutants of *Neurospora crassa*. *Plant Physiology* **68**, 745-749.
- KINNAIRD, J. H., KEIGHREN, M. A., KINSEY, J. A., EATON, M. & FINCHAM, J. R. S. (1982). Cloning of the *am* (glutamate dehydrogenase) gene of *Neurospora crassa* through the use of a synthetic DNA probe. *Gene* **20**, 387-396.
- LAUTER, F.-R. & RUSSO, V. E. A. (1990). Light-induced dephosphorylation of a 33 kDa protein in the wild-type strain of *Neurospora crassa*: the regulatory mutants *wc-1* and *wc-2* are abnormal. *Journal of Photochemistry and Photobiology* **5**, 95-103.
- LAUTER, F.-R. & RUSSO, V. E. A. (1991). Blue light induction of conidiation-specific genes in *Neurospora crassa*. *Nucleic Acids Research* **19**, 6883-6886.
- MÜLLER, B. & RUSSO, V. E. A. (1989). Nitrogen starvation or glucose limitation induces conidiation in constantly shaken liquid cultures of *Neurospora crassa*. *Fungal Genetics Newsletters* **36**, 58-60.
- NAWRATH, C. & RUSSO, V. E. A. (1990). Fast induction of translatable mRNA by blue light in *Neurospora crassa* wildtype: the *wc-1* and *wc-2* mutants are blind. *Journal of Photochemistry and Photobiology* **4**, 261-271.
- NELSON, M. A., MORELLI, G., CARATTOLI, A., ROMANO, N. & MACINO, G. (1989). Molecular cloning of a *Neurospora crassa* carotenoid biosynthetic gene (albino-3) regulated by blue light and the products of the white collar genes. *Molecular and Cellular Biology* **9**, 1271-1276.
- NELSON, R. E., SELITRENNIKOFF, C. P. & SIEGEL, R. W. (1975) Results and Problems in Cell Differentiation, pp. 291-310. Edited by J. Reinert and H. Holzer. Berlin: Springer.
- ORBACH, M. J., PORRO, E. B. AND YANOFSKY, C. (1986). Cloning and characterization of the gene for β -tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. *Molecular and Cellular Biology* **6**, 2452-2461.
- RICCI, M., KRAPPANN, D. & RUSSO, V. E. A. (1991). Nitrogen and carbon starvation regulate conidiation and protoperithecia formation in *Neurospora crassa* grown on solid medium. *Fungal Genetics Newsletters* **38**, 87-88.
- ROBERTS, A. N., BERLIN, V., HAGER, K. & YANOFSKY, C. (1988). Molecular analysis of a *Neurospora crassa* gene expressed during conidiation. *Molecular and Cellular Biology* **8**, 2411-2418.
- RUSSO, V. E. A. (1986) Are carotenoids the blue light photoreceptor in the photoinduction of protoperithecia in *Neurospora crassa*? *Planta* **168**, 56-60.
- RUSSO, V. E. A. (1988). Blue light induces circadian rhythms in the *bd* mutant of *Neurospora*: double mutants *bd,wc-1* and *bd,wc-2* are blind. *Journal of Photochemistry and Photobiology* **2**, 59-65.
- RUSSO, V. E. A., POHL, U. & VOLPI, L. (1981). Carbon dioxide inhibits photogenesis in *Phycomyces* and blue light overcomes this inhibition. *Photochemistry and Photobiology* **34**, 233-236.
- SACHS, M. S. & YANOFSKY, C. (1991). Developmental expression of genes involved in conidiation and aminoacid starvation in *Neurospora crassa*. *Developmental Biology* **148**, 117-128.
- SARGENT, M. L. & BRIGGS, W. R. (1967). The effects of light on a circadian rhythm of conidiation in *Neurospora*. *Plant Physiology* **42**, 1504-1510.
- SARGENT, M. L. & KALDENBORN. (1972). Effects of medium composition and carbon dioxide on circadian conidiation in *Neurospora*. *Plant Physiology* **50**, 171-175.
- SARGENT, M. L., BRIGGS, W. R. & WOODWARD, D. O. (1966). Circadian nature of a rhythm expressed by an invertaseless strain of *Neurospora crassa*. *Plant Physiology* **41**, 1343-1349.
- SCHMIDHAUSER, T. J., LAUTER, F.-R., RUSSO, V. E. A. & YANOFSKY, C. (1990). Cloning, sequence, and photoregulation of *al-1*, a carotenoid biosynthetic gene of *Neurospora crassa*. *Molecular and Cellular Biology* **10**, 5064-5070.
- SIEGEL, R. W., MATSUYAMA, S. S. & UREY, J. C. (1968). Induced macroconidiation formation in *Neurospora crassa*. *Experientia* **24**, 1179-1181.
- SOKOLOVSKY, V. Y., KALDENHOFF, R., RICCI, M. & RUSSO, V. E. A. (1990). Fast and reliable mini-prep RNA extraction from *Neurospora crassa*. *Fungal Genetics Newsletters* **37**, 39-40.
- SOMMER, T., DEGLI INNOCENTI, F. & RUSSO, V. E. A. (1987). Role of nitrogen in the photoinduction of protoperithecia and carotenoids in *Neurospora crassa*. *Planta* **170**, 205-208.
- SOMMER, T., CHAMBERS, J. A. A., EBERLE, J., LAUTER, F.-R. & RUSSO, V. E. A. (1989). Fast light-regulated genes *Neurospora crassa*. *Nucleic Acids Research* **17**, 5713-5723.
- SPRINGER, M. L. & YANOFSKY, C. (1989). A morphological and genetic analysis of the conidiophore development in *Neurospora crassa*. *Genes and Development* **3**, 559-571.
- TURIAN, G. (1977). Fungal differentiation. In *Biotechnology and Fungal Differentiation*, pp. 1-15. Edited by J. Meyrath & J. D. Bu'Lock. London: Academic Press.