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Light regulation of the 22 kd heat shock gene transcription and its translation product accumulation in *Chlamydomonas reinhardtii*

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Expression of the nuclear coded heat shock protein HSP22 in *Chlamydomonas reinhardtii y-1* cells is light regulated at the level of transcript accumulation. In dark grown cells, containing a non-differentiated plastid, light has an additional regulatory effect on the accumulation of HSP22. When such cells are exposed to heat stress in the light, poly(A)$^+$ RNA hybridizing with the HS22 probe is synthesized at levels comparable with those found in cells pre-illuminated for 3 h (greening) prior to the heat shock. However, this RNA is poorly translated *in vitro* and HSP22 does not accumulate *in vivo*. HS22 mRNA efficiently translated *in vitro* is induced in dark grown cells only when chloroplast differentiation has been initiated by exposure to the light for 3 h. In these cells HSP22 accumulates during heat shock. Inhibition of plastid translation activity during light-dependent chloroplast development prevents accumulation of HSP22 *in vivo*. However the HS22 mRNA formed in this case can be efficiently translated *in vitro*. Light requirement for the accumulation of HSP22 during heat stress is exhibited also by wild type *C.reinhardtii* cells which possess a differentiated chloroplast irrespective of the light conditions during cell growth. However dark grown wild type cells do not require pre-illumination for developing the ability to accumulate HSP22 during heat stress in the light.

Key words: Chlamydomonas/gene expression/heat shock proteins/light regulation/mRNA maturation

Introduction

Heat stress induces expression of nuclear encoded genes in all eukaryotic organisms investigated so far (Kelly and Schlesinger, 1978; Nagao *et al.*, 1985; Lindquist, 1986). In plants, several classes of heat shock proteins (HSPs) have been identified. These include proteins in the molecular weight range of 60-110 kd, 30-50 kd, as well as a group of low molecular mass proteins in the range of 15-30 kd. The low molecular mass HSPs encoded by nuclear multigene families are very complex and abundant in plants (Key *et al.*, 1983; Nover and Scharf, 1984; Lindquist, 1986; Schoffl *et al.*, 1986). Some of these proteins are translocated into the chloroplast (Kloppstech *et al.*, 1985; Suss and Yordanov, 1986; Vierling *et al.*, 1986). The translocation of nuclear encoded HSPs to the chloroplast involves processing of the precursor proteins synthesized in the cytosol (Kloppstech *et al.*, 1985; Vierling *et al.*, 1986, 1988). The 26 kd HSPs in pea were found to be processed when transported *in vitro* into chloroplasts isolated from both, control and heat stressed plants. However, association of the pea HSP22 with the thylakoid membranes, occurred only when the chloroplasts used for transport *in vitro* were isolated from heat stressed plants (Kloppstech *et al.*, 1985; Glaczinski and Kloppstech, 1988).

It was reported before that in the green alga *Chlamydomonas reinhardtii y-1* nuclear encoded heat shock protein, HSP22, appears to be associated with the chloroplast membranes *in vivo* (Kloppstech *et al.*, 1985). Since the presence of heat shock proteins correlated with a transient resistance to the synergistic adverse effect of light and heat stress on photosynthetic activity (Schuster *et al.*, 1988) it was of interest to assay the effect of light on the synthesis and accumulation of HSP22 in these cells.

The results of this work demonstrate that accumulation of HS22 mRNA is light regulated in *Chlamydomonas*. In the *y-1* mutant cells grown in the dark in which the plastid is not fully differentiated, an additional, indirect effect on the accumulation of HSP22 is found which can be attributed to a post-transcriptional control dependent on the light induced chloroplast differentiation.

Results

Cells heat stressed in the dark do not accumulate HSP22

Accumulation of the HSP22 can be demonstrated in total cell protein extract by immunodecoration of SDS-PAGE resolved polypeptides following transfer to nitrocellulose paper. The time course of HSP22 accumulation is shown in Figure 1A which shows that HSP22 can be already detected after ~30 min and reaches a plateau after ~2-3 h of heat stress. However, HSP22 cannot be detected in light grown cells when exposed to heat stress in the dark, even after 2 h of incubation (Figure 1, B and C). This result was obtained with both wild type cells (which possess a differentiated chloroplast when grown either in the light or dark) and the *y-1* mutant cells. The *y-1* mutant is not distinguishable from the wild type cells when grown in the light. Dark grown *y-1* cells lack a differentiated chloroplast due to their inability to synthesize chlorophyll in absence of light (Ohad *et al.*, 1967). The light requirement is specific for the accumulation of HSP22. The synthesis and accumulation of other heat shock proteins during heat shock, as detected by ^{35}S radioactive labelling, continues in the dark as in the light (data not shown). These results could indicate either that HSP22 is not synthesized or it is unstable in cells heat stressed in the dark.

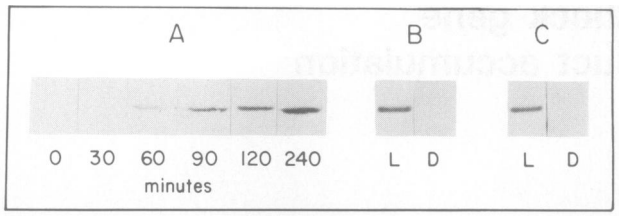

Fig. 1. Accumulation of HSP22 during heat shock in *y-1* and wild type cells. Cells were heat treated at 42°C in the light (10 W/m^2) or in the dark for times as indicated. Samples were taken and total cell protein was resolved by SDS–PAGE. Proteins were transferred to nitrocellulose paper and HSP22 was identified by immunodecoration using anti-HSP22 serum and alkaline phosphatase for detection. (**A**) accumulation of HSP22 as a function of time in light exposed *y-1* cells; (**B**) accumulation of HSP22 in *y-1* cells exposed to the light or dark for 2 h; (**C**) accumulation of HSP22 in wild type cells exposed to the light or dark; L, light, D, dark.

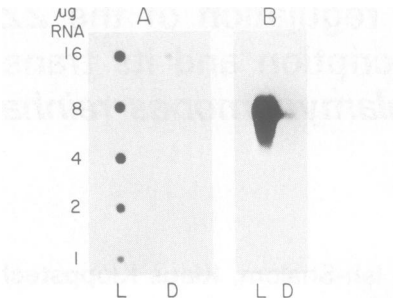

Fig. 2. Transcript levels of HS22 gene in cells heat treated in the light (L) or dark (D). Total (A), or poly(A)$^+$ (B), RNA was extracted from cells heat treated at 42°C for 2 h. (**A**) different amounts of RNA were applied onto a nitrocellulose filter and the immobilized RNA was probed with ^{32}P-labelled HS22 cDNA clone; (**B**) 1 μg mRNA was fractionated on a formaldehyde–agarose gel, transferred to nitrocellulose paper and hybridized as in (A).

The HSP22 transcript does not accumulate in cells exposed to heat stress in the dark

Since light affects chloroplast development in the *y-1* mutant cells, the effect of light on the expression of HSP22 was further investigated in this mutant. To detect at what step light affects HSP22 accumulation, the level of its transcript was assayed in cells heat shocked in the light or dark. Results of such an experiment are shown in Figure 2A and demonstrate that the HSP22 transcript levels are 15- to 20-fold lower in total RNA isolated from dark treated cells as compared with those heat shocked in the light. The transcript detected in the dark seems to be identical in molecular size to that of the transcript synthesized in the light as indicated by the Northern blot of poly(A)$^+$ RNA isolated from cells heat treated in the light or in the dark (Figure 2B).

The low level of HSP22 mRNA in cells heat treated in the dark could be due to an overall reduction in transcription activity under these conditions. To test this possibility, poly(A)$^+$ RNA isolated from cells heat stressed in the dark or in the light was translated *in vitro*. The level of the HSP22 translated from limiting amounts of RNA, isolated from dark treated cells, was considerably lower than that of the light control sample. Other heat shock proteins were translated to similar extents when using RNA isolated from both light or dark treated cells (Figure 3, open arrows). Thus the low level of HSP22 in cells heat stressed in the dark is due to a specific reduction in HS22 transcript level and not to a general lowering of transcription activity.

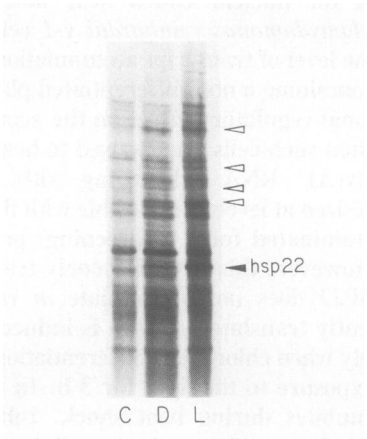

Fig. 3. *In vitro* translation of poly(A)$^+$ mRNA from cells heat treated in the light (L) or dark (D). Cells were heat treated at 42°C or incubated at 25°C (control, C) for 2 h and mRNA was extracted and translated as described. [^{35}S]methionine-labelled translation products were resolved by SDS–PAGE and autoradiographed; black arrowhead, HSP22, translated preferentially in the light; open arrowheads, HSPs translated about equally in the light and dark.

HSP22 does not accumulate in dark grown y-1 mutant cells containing a non-differentiated plastid

Data so far presented indicate that light affects the induction of the nuclear encoded HSP22. The question arises whether the developmental stage of the chloroplast which is light dependent, may affect the regulation of HSP22 accumulation. The *Chlamydomonas y-1* mutant used in this work does not accumulate chloroplast membranes in the dark due to a block in the pathway of chlorophyll synthesis (Ohad *et al.*, 1967). In dark grown cells the chloroplast is only partially differentiated and lacks the thylakoid membranes which develop upon exposure of the cells to the light (greening; Ohad, 1975).

Dark grown *y-1* cells do not accumulate significant amounts of HSP22 when heat shocked in the light before or during the initial stage of the greening process (Figure 4). The appearance of the HSP22 does not coincide with increase in the amount of chlorophyll but correlates with the onset of photosynthetic activity of photosystem II, measured as a rise in variable fluorescence (Schuster *et al.*, 1988), (Figure 4). This behaviour of HS22 is quite different from other light regulated nuclear genes such as *cab*. The transcript of this gene(s) is not detected in dark grown cells by *in vitro* translation and appears after ~3 h of greening. However translatable transcript is accumulated when the dark grown cells are heat treated (38°C) in the dark prior to the greening (Hoober *et al.*, 1982).

The induction of HS22 gene(s) transcript is constant during the greening process of y-1 cells

Accumulation of HSP22 induced by heat shock as a function of pre-illumination of dark grown cells (Figure 4), could be due to a rise in the HS22 gene transcript level or to post-transcriptional control. Light treatment of the cells prior to heat stress did not affect the heat induced HS22 transcript level (Figure 5). Furthermore, inhibition of chloroplast translation activity, known to block chloroplast differentiation in greening cells (Ohad, 1975), did not affect the HS22 transcript level (Figure 5). Northern blots of RNA isolated from cells heat stressed after various pre-illumination times

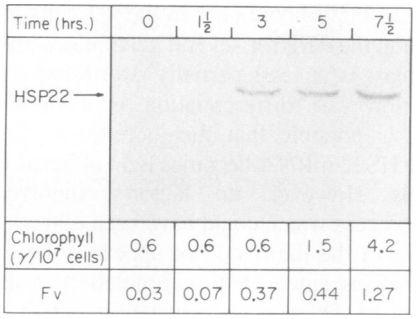

Fig. 4. Induction of HSP22 during the greening process. Cells were grown in the dark for five to six generations (degreening). The cells were exposed to light for the times indicated and subsequently heat treated for 2 h (42°C, 10 W/m^2). A fraction enriched in chloroplast membranes was isolated (Kloppstech *et al.*, 1985). The polypeptides were resolved by SDS−PAGE followed by immunodecoration with anti-HSP22 and alkaline phosphatase.

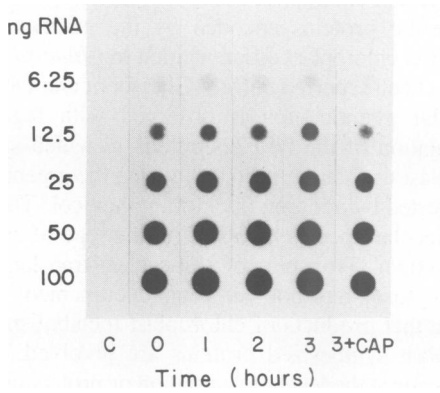

Fig. 5. HS22 transcript levels are not affected by the plastid differentiation stage. Cells were grown in the dark for five to six generations. The dark grown cells were exposed to the light (greening) for time as indicated. Samples were heat treated for 45 min at 42°C in the light (10 W m^2). Poly(A)$^+$ RNA was extracted, dot-blotted on nitrocellulose paper and probed with a [^{32}P]cDNA HS22 clone; C: RNA from control cells not exposed to heat shock; 3+CAP: RNA from cells exposed to the light for 3 h in the presence of chloramphenicol (200 μg/ml) added to prevent plastid translation activity during the greening.

indicate the presence of equal amounts of HS22 mRNA, having the same electrophoretic mobility, in all samples (data not shown).

Thus differences in accumulation of HSP22 following increasing pre-illumination time, could not be due to different transcript levels but to a post-transcription RNA modification or to a translation control. To distinguish between these possibilities, *in vitro* translation of mRNA obtained from dark grown cells exposed to the light for increasing times prior to the heat treatment, as in Figure 5, was assayed. The accumulation of HSP22 in these cells was also quantitated by immunoblotting. The results, (Figure 6A), show low levels of HSP22 in cells exposed to light for 0−1 h before heat stress, followed by an increase in the protein level in cells pre-illuminated for 2 and 3 h respectively. Similar results were obtained when RNA samples from the same cells were translated *in vitro* and immunoprecipitated (Figure 6B and C) indicating that the RNA induced in the initial phase of light treatment is less translatable than that of the later phase.

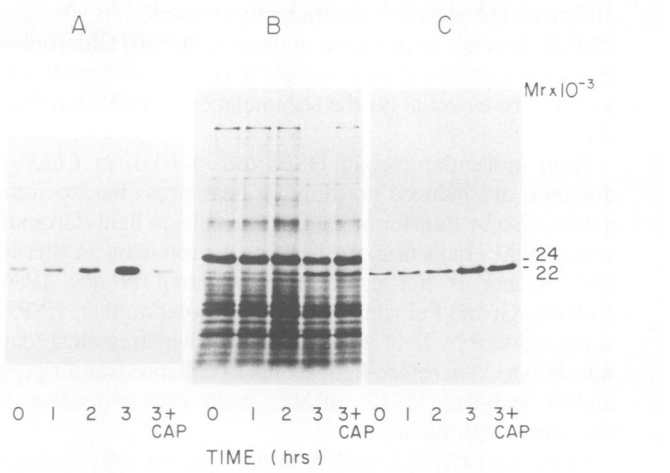

Fig. 6. Changes in the translation properties and in the accumulation of translation products of HS22 RNA during light-induced plastid development. Dark grown cells were greened in the light in the absence of chloramphenicol (200 μg/ml). At the times indicated cells were heat treated at 42°C for 45 min at a light intensity of 10 W/m^2 and samples were taken for total protein and poly(A)$^+$ RNA extraction. (**A**) Western blot of total cell protein; HSP22 was detected by ^{125}Iodinated protein A; (**B**) autoradiogram of *in vitro* translated products using poly(A)$^+$ RNA obtained from the same cells; (**C**) immunoprecipitated HSP22 from the translation mixture of B.

The level of HSP22 induced in cells greening in the presence of chloramphenicol was as low as that induced in the dark grown cells (Figure 6A). The level of HSP22 mRNA extracted from these cells was similar to that of the cells exposed for 3 h to light (Figure 5). Furthermore, this RNA could be translated efficiently *in vitro* as shown by immunoprecipitation of its translation products (Figure 6B and C).

These results indicate the presence of yet an additional control step. Light induced chloroplast differentiation in *Chlamydomonas* cells requires synthesis of many chloroplast translated proteins, both, soluble and membrane bound. Since chloroplast translation is sensitive to chloramphenicol, the effect of this antibiotic on the *in vivo* translation/accumulation of the cytosolic HSP22 implies that the low level of HSP22 in the chloramphenicol treated cells could be ascribed to control of its accumulation mediated by chloroplast synthesized proteins.

Discussion

Light regulation at the transcription level has repeatedly been demonstrated for expression of various nuclear genes encoding chloroplast localized proteins (Apel and Kloppstech, 1978; Silverthorne and Tobin, 1987). Furthermore, the developmental stage of the chloroplast which is in itself light regulated seems to affect the expression of these genes (Mayfield and Taylor, 1984; Batschauer *et al.*, 1986; Oelmuller and Mohr, 1986; Stockhaus *et al.*, 1989). This type of regulation includes at least one additional nuclear coded protein which is not localized in the chloroplast (Oelmuller *et al.*, 1988). Three levels of regulation affected by light have now been found to occur for a nuclear coded heat shock protein, HSP22, considered to be associated with the chloroplast (Grimm *et al.*, 1989). (i) Light controls the accumulation of HS22 gene transcript. (ii) The transcript formed in cells grown in the dark and containing a non-

differentiated plastid is inefficiently translated *in vitro* and HSP22 does not accumulate in these cells. (iii) Chloroplast translation activity during its light dependent differentiation seems to be essential for the accumulation of HSP22 in these cells.

High molecular weight HSPs (68−80 kd) in *Chlamydomonas* are induced not only by heat stress but, to some extent, also by transfer of dark grown cells to light (Gromoff *et al.*, 1989). Induction of a heat shock protein by an alternative inducer is not uncommon, (Mestril *et al.*, 1986; Riddihough and Pelham, 1986). As opposed to that, HSP22 is not induced by light in the absence of heat treatment (data not shown). Therefore light seems to enhance transcription and/or stabilize HS22 mRNA, both effects leading to transcript accumulation.

Induction of HS22 mRNA in dark grown cells having a non-differentiated plastid results in the accumulation of comparable amounts of transcript independent of the pre-illumination (greening) time. However accumulation of the HS22 mRNA induced during the initial period of greening (0−2 h) did not result in a comparable accumulation of the heat shock protein. This could indicate that this RNA may not be efficiently translated *in vivo* as indeed is demonstrated by its poor translation *in vitro* using the wheat germ system. The fact that this system translated efficiently the RNA obtained from cells after 3 h of illumination implies that the difference in the translation ability of the RNA resides in the HS22 mRNA proper. This could be due to incomplete maturation arrested at various steps of this process. Since the molecular weight of the HS22 mRNA as estimated from Northern blots is indistinguishable from that of mature, translatable RNA (data not shown) and since this RNA was isolated by binding to oligo(dT) columns, it seems unlikely that maturation in the initial stages of greening was arrested at the adenylation step. Although CAPing occurs prior to adenylation, it is possible that the translation of the HS22 gene transcript induced at the early phase of greening is impaired due to incomplete CAP methylation (Caldwell and Emerson, 1985). Another possibility one could consider, is that the splicing of the HS22 transcript elicited by heat stress during the initial phase of the greening process is impaired. Incomplete splicing resulting in formation of an mRNA only slightly different in its molecular weight as compared with mature mRNA (for review, see Green, 1989) could remain undetected by the Northern blotting system used in this work.

It has been demonstrated before that the system responsible for mRNA splicing in eukaryotic organisms is inactivated in heat treated cells (Yost and Lindquist, 1986). The inactivation of the splicing system was ascribed to the inactivation of a protein factor required for the formation of spliceosomes (Bond, 1988).

A similar effect was also demonstrated for the maturation of the chloroplast encoded *psaA* gene, impaired by heat shock in *Chlamydomonas* cells (Choquet *et al.*, 1988).

If splicing of intron-containing genes is blocked in heat treated cells, one should assume either that heat shock genes expressed during the temperature rise do not contain introns (Lindquist, 1986; Bond, 1988) or alternatively, splicing of intron-containing heat shock genes implies the presence of a heat resistant splicing system. The putative maturation of HS22 mRNA could be related to a specific splicing system which might be under light/chloroplast control. This type of control can be detected only in the *y-1* cells which have been grown in the dark for several generations and in which the chloroplast is at least partially dedifferentiated.

Since chloroplast differentiation is a light regulated process, it is possible that the increase in the level of translatable HS22 mRNA becomes light affected in the dark grown cells. However, the factor(s) involved in this regulatory process which could have been diluted out during cell division in the dark, do not appear to be chloroplast encoded proteins since their accumulation in the light is independent of chloroplast translation activity. Factors regulating nuclear gene expression originating in the chloroplast which might not be of a protein nature have been suggested before (Oelmuller *et al.*, 1986). In agreement with this hypothesis, wild type *Chlamydomonas* cells which possess a differentiated chloroplast when grown in the dark and require light for the accumulation of the HSP22 as well, do not show this type of light requirement for the expression of HS22 (data not shown).

An effect of chloroplast translation activity on the synthesis of cytosolic proteins encoded by the nuclear gene *cab*, involved in chloroplast differentiation in *Chlamydomonas y-1* cells has been reported before (Ghershoni and Ohad, 1980). A similar phenomenon is observed with regard to the accumulation of the HSP22 in cells in which synthesis of chloroplast translation products during the greening process was arrested by addition of chloramphenicol. The nature of the molecular species involved in this type of control is as yet unknown. Transport of chloroplast translated proteins to the cytosol has not yet been documented. Thus it is possible that products of chloroplast metabolism requiring chloroplast synthesized proteins are involved. The effect could occur at the level of translation or protection of HSP22 from degradation.

The understanding of the mechanism whereby light regulates the transcript level of HS22 and indirectly, its ability to be translated into stable translation products rest upon the isolation of HS22 gene and elucidation of its structure. Work aimed at this goal is now being initiated in our laboratories.

Materials and methods

Cell culture and greening of dark grown cells

Chlamydomonas reinhardtii wild type and *y-1* cells were grown in the light in a mineral medium containing acetate as a carbon source as described before (Ohad *et al.*, 1967). To obtain cells containing a non-differentiated plastid the cells were grown in the dark for 5−6 days (Ohad, 1975). Cells were harvested by centrifugation, washed once in fresh growth medium and resuspended in the same medium at a final concentration of about $1-2 \times 10^7$ cells/ml equivalent to 30−50 μg chlorophyll/ml in the light grown cells. The *y-1* mutant does not synthesize chlorophyll and photosynthetic membranes in the dark. These are diluted during cell division to <1−2% of the original level and resynthesized if the cells are exposed to the light [25 W/m^2, greening, (Ohad *et al.*, 1967)].

Heat treatment and immunodetection of HSP22

Heat shock was carried out in the light as described before (Kloppstech *et al.*, 1985).

For immunodetection of HSP22 samples were taken, (1−2 ml), the cells were pelleted by centrifugation in an Eppendorf microfuge and extracted with 1 ml of 90% acetone to remove pigments and lipids. The denatured proteins were solubilized in sample buffer and resolved by SDS−PAGE as described (Laemmli, 1970, or Neville, 1971). The resolved polypeptides were transferred to nitrocellulose paper and immunodecorated with anti-HSP22 antibodies (Grimm *et al.*, 1989) detected by [^{125}I] protein A or alkaline phosphatase.

Extraction of RNA and identification of HS22 mRNA

Total cell RNA was extracted as described before (Chirgwin, 1979). For preparation of poly(A)$^+$ RNA the extraction procedure described by Kloppstech *et al.* (1985) was used. Detection of HS22 mRNA was carried out by hybridization with ^{32}P-labelled HS22 cDNA obtained from a cDNA clone (Grimm *et al.*, 1989). Electrophoretic separation of HS22 RNA was carried out in formaldehyde–agarose gels followed by transfer to nitrocellulose paper.

In vitro translation of poly(A)$^+$ RNA

Translation *in vitro* was carried out using the wheat germ system as described (Roberts *et al.*, 1973). The translation products were resolved by SDS–PAGE according to Neville (1971), and fluorographed. The heavy labelled band at ~25 kd present in all translation experiments is identified as the precursor of the small subunit of ribulose-1,5-bisphosphate carboxylase, (Kloppstech *et al.*, 1985) whose mRNA is constant and abundant in both light and dark grown *Chlamydomonas* cells.

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References

Apel,K. and Kloppstech,K. (1978) *Eur. J. Biochem.*, **85**, 581–588.
Batschauer,A., Mosinger,E., Kreuz,K., Dorr,I. and Apel,K. (1986) *Eur. J. Biochem.*, **154**, 625–634.
Bond,U. (1988) *EMBO J.*, **7**, 3509–3518.
Caldwell,D.C. and Emerson,C.P.,Jr (1985) *Cell*, **42**, 691–700.
Chirgwin,J.M., Prsybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry*, **18**, 5294–5299.
Choquet,Y., Goldschmidt-Clermont,M., Girard-Bascou,J., Kuck,U., Bennoun,P. and Rochaix,J.-D. (1988) *Cell*, **52**, 903–913.
Ghershoni,J.M. and Ohad,I. (1980) *J. Cell Biol.*, **86**, 124–131.
Glaczinski,H. and Kloppstech,K. (1988) *Eur. J. Biochem.*, **173**, 579–583.
Green,M.R. (1989) *Curr. Opinion Cell Biol.*, **1**, 519–525.
Grimm,B., Ish-Shalom,D., Even,D., Glaczinski,H., Ottersbach,P., Ohad,I. and Kloppstech,K. (1989) *Eur. J. Biochem.*, **182**, 539–546.
Gromoff,E.D., Treier,U. and Beck,C.F. (1989) *Mol. Cell. Biol.*, **9**, 3911–3918.
Hoober,J.K., Marks,D.B., Keller,B.J. and Margulies,M.M. (1982) *J. Cell Biol.*, **95**, 552–558.
Kelly,P. and Schlesinger,M.J. (1978) *Cell*, **15**, 1277–1286.
Key,J.L., Czarnecka,E., Lin,C.Y., Kimple,J.A., Mothershed,C. and Schoffl,F. (1983) In Randoll,D.D., Blevins,D.J., Larson,R.L. and Rapp,B.J. (eds), *Current Topics in Plant Biochemistry and Physiology*. Columbia, MO, pp. 107–118.
Kloppstech,K., Meyer,G., Schuster,G. and Ohad,I. (1985) *EMBO J.*, **4**, 1901–1909.
Laemmli,U.K. (1970) *Nature*, **227**, 680–685.
Lindquist,S.L. (1986) *Annu. Rev. Biochem.*, **55**, 1151–1191.
Mayfield,S.P. and Taylor,W.C. (1984) *Eur. J. Biochem.*, **144**, 79–84.
Mestril,R., Schiller,P., Amin,J., Klepper,H., Anathan,J. and Voellmy,R. (1986) *EMBO J.*, **7**, 1667–1673.
Nagao,R.T., Czarnecka,E., Gurley,W.B., Schoffl,F. and Key,J.L. (1985) *Mol. Cell., Biol.*, **5**, 3417–3428.
Neville,D.M., Jr (1971) *J. Biol. Chem.*, **246**, 6328–6334.
Nover,L. and Scharf,K.D. (1984) *Eur. J. Biochem.*, **139**, 303–313.
Oelmuller,R. and Mohr,H. (1986) *Planta*, **167**, 106–113.
Oelmuller,R., Lavitan,I., Bergfeld,R., Rajasekhar,V.K. and Mohr,H. (1986) *Planta*, **168**, 482–492.
Oelmuller,R., Schuster,C. and Mohr,H. (1988) *Planta*, **174**, 75–83.
Ohad,I. (1975) In Tzagaloff,A. (ed.) *Membrane Biogenesis, Mitochondria, Chloroplast and Bacteria*. Plenum Press, New York, pp. 279.
Ohad,I., Siekevitz,P. and Palade,G.E. (1967) *J. Cell Biol.*, **35**, 553–584.
Riddihough,G. and Pelham,R.B. (1986) *EMBO J.*, **7**, 1653–1658.
Roberts,B.E. and Peterson,B.M. (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 2330–2334.
Schoffl,F., Boumann,G., Raschke,E. and Bevan,M. (1986) *Phil. Trans. R. Soc. Lond.*, **B 314**, 453–468.
Schuster,G., Even,D., Kloppstech,K. and Ohad,I. (1988) *EMBO J.*, **7**, 1–6.
Silverthorne,J. and Tobin,M.E. (1987) *Bioassays*, **7**, 18–23.
Stockhaus,J., Schell,J. and Willmitzer,L. (1989) *EMBO J.*, **8**, 2445–2451.
Suss,K.H. and Yordanov,I.T. (1986) *Plant Physiol.*, **81**, 192–200.
Vierling,E., Mishkind,M.L., Schmidt,G.W. and Key,J.L. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 361–365.
Vierling,E., Nagao,R.T., DeRocher,A.E. and Harris,L.M. (1988) *EMBO J.*, **7**, 575–581.
Yost,H.J. and Lindquist,S. (1986) *Cell*, **45**, 185–193.

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