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Organization and regulation of zein genes in maize endosperm

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[Plate 1]

Zein, the major storage protein of maize endosperm, is constituted by a group of similar polypeptides encoded by a multigene family. The structural genes are located into three main clusters on chromosomes 4, 7 and 10. The rate of accumulation of zein polypeptides is under the control of several positive regulatory loci. The mutant alleles at these loci (*O2*, *O6*, *O7*, *Fl2*, *De-B30*, *Mc*) reduce more or less drastically the rate of zein deposition. By analysing the interactions among the mutants, epistatic, additive and synergistic effects were observed indicating the existence of multiple pathways controlling zein deposition. Proteins, other than zeins, associated with the *O2*, *O6* and *Fl2* loci have been identified and characterized.

Introduction

Zeins are a group of proteins accumulated for storage purposes in maize endosperm during seed development. In mature seeds, they represent more than 60% of the total endosperm proteins. For several reasons the zein system is a favourable model system for the molecular analysis of gene arrangement in the chromosomes and of gene regulation: zeins are a family of related polypeptides encoded by a multigene family; the structural genes are clustered into three main sites of the maize genome; all the zein genes are synchronously expressed and several mutants are known that affect the onset or the rate of zein deposition (Soave & Salamini 1982). A further incentive for the study of the genetics and physiology of zeins is the poor nutritional value of the maize proteins because of their deficiency in the essential amino acids lysine and tryptophan. This is because of the very low content of these amino acids in zeins.

Molecular and cytological features

Zeins are hydrophobic proteins soluble in alcoholic solutions in the presence of reducing agents. By using sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis of reduced zein proteins, up to ten components can be separated with an apparent molecular mass ratio (M_r) of 23000, 22000 and 21000 (collectively termed the 22 kDa class), 20000, 19000 and 18000 (20 kDa class), 15000 and 14000 (14 kDa class), and 12000 and 10000 (10 kDa class) (figure 1, plate 1). The 22 and 20 kDa classes represent together 70–80% of total zeins and show extensive charge heterogeneity on isoelectricfocusing (i.e.f.) in polyacrylamide gels, while the two lower M_r classes give only single i.e.f. bands (Lee *et al.* 1976; Righetti *et al.* 1977). By two dimensional electrophoretic analysis up to 28 individual polypeptides can be identified (Hagen & Rubenstein 1980; Vitale *et al.* 1980; Burr & Burr 1981). Data from DNA sequencing, dot hybridizations and restriction enzyme maps of complementary DNA (cDNA) or genomic

zein DNA clones indicate an overall homology among the higher M_r zein components with a sequence of approximately 20 amino acids repeated up to nine times in the polypeptide chain (Pedersen *et al.* 1982; Marks & Larkins 1982). The two lower M_r classes are similar in amino acid composition to the other zeins although they contain significantly more methionine and cysteine (Gianazza *et al.* 1977; Melcher 1979). Evidence suggests that each zein polypeptide is the product of a specific structural gene. Since estimates of the number of genes in the zein family indicate more than 100 genes of related sequence (Viotti *et al.* 1979; Wienand & Feix 1980; Hagen & Rubenstein 1981), it is possible that some of these could be silent. Unlike many other eukaryotic genes, zein genes do not contain intervening sequences although the flanking regions contain sequences similar to the putative transcriptional signals present in other eukaryotes (Wienand *et al.* 1981; Pedersen *et al.* 1982; Spena *et al.* 1982). By hybridization of electrophoretically fractionated total maize endosperm RNA with higher M_r zein cDNA clones, mature zein mRNA of about 1000 bases (with a 100–120 poly(A)tail) and putative mRNA precursors of about 1800, 2800 and 3800 bases have been identified (Burr *et al.* 1982; Langridge *et al.* 1982*a*). Zein RNAs are translated by ribosomes attached to endoplasmic reticulum (e.r.) into preproteins (about 2000 Da larger than mature zeins) (Burr & Burr 1976, 1981). The signal peptide is cleaved co-translationally and the nascent proteins secreted into the lumen of the e.r. where they form large aggregates producing localized enlargements along the e.r. (the so-called protein bodies). Apparently there is no asymmetric distribution of zein synthesizing sites along the e.r. membranes (Larkins & Hurkmann 1978). During the development of the maize endosperm, zein synthesis starts around 15 d after pollination and all the polypeptides are accumulated coordinately at the same rate for more than 25 d (Soave & Salamini 1983).

Genetic organization of zein genes

Formal genetics

After an extensive analysis of zeins from several inbred lines of maize, a great variability in the i.e.f. and SDS polyacrylamide gel banding pattern was observed. The genetical bases of this variability was inferred from inheritance studies of crosses between inbreds differing in the presence or absence of some zein i.e.f. bands (Soave *et al.* 1981*a*). In the seeds of the two reciprocal F_1 generations (maize endosperm is triploid with two of the genomes contributed by the maternal parent), the presence of a band is dominant over its absence and the amount of the component in the F_1s correlates to the gene dose present. Moreover, when seeds from the F_2 generation are analysed, a simple segregation is observed for the presence or absence of those particular zein i.e.f. bands specifically contributed by the parents. On this basis, a search for the linkage relations and the chromosomal location of the zein structural genes encoding for some zein polypeptides was undertaken. Up to now 20 genes have been identified and mapped (figure 2). They have been given the symbol *Zp* (standing for zein polypeptide) followed by two numbers, the first indicating the M_r (in 1000 s) of the corresponding polypeptide and the second its position in the standard i.e.f. zein pattern (Bonanomi *et al.* 1983). Therefore the gene *Zp20/1* indicates a gene encoding for a zein polypeptide with a M_r of 20000 and occupying position 1 in the zein i.e.f. pattern (the most alkaline position). Among the 20 genes mapped, ten are on chromosome 4 (eight on the short arm and two on the long arm); nine on the short arm of chromosome 7 and one on the long arm of chromosome 10 (Valentini *et al.* 1979; Soave *et al.* 1981*a*, 1982). This genetic organization is of interest from several points of view. First,

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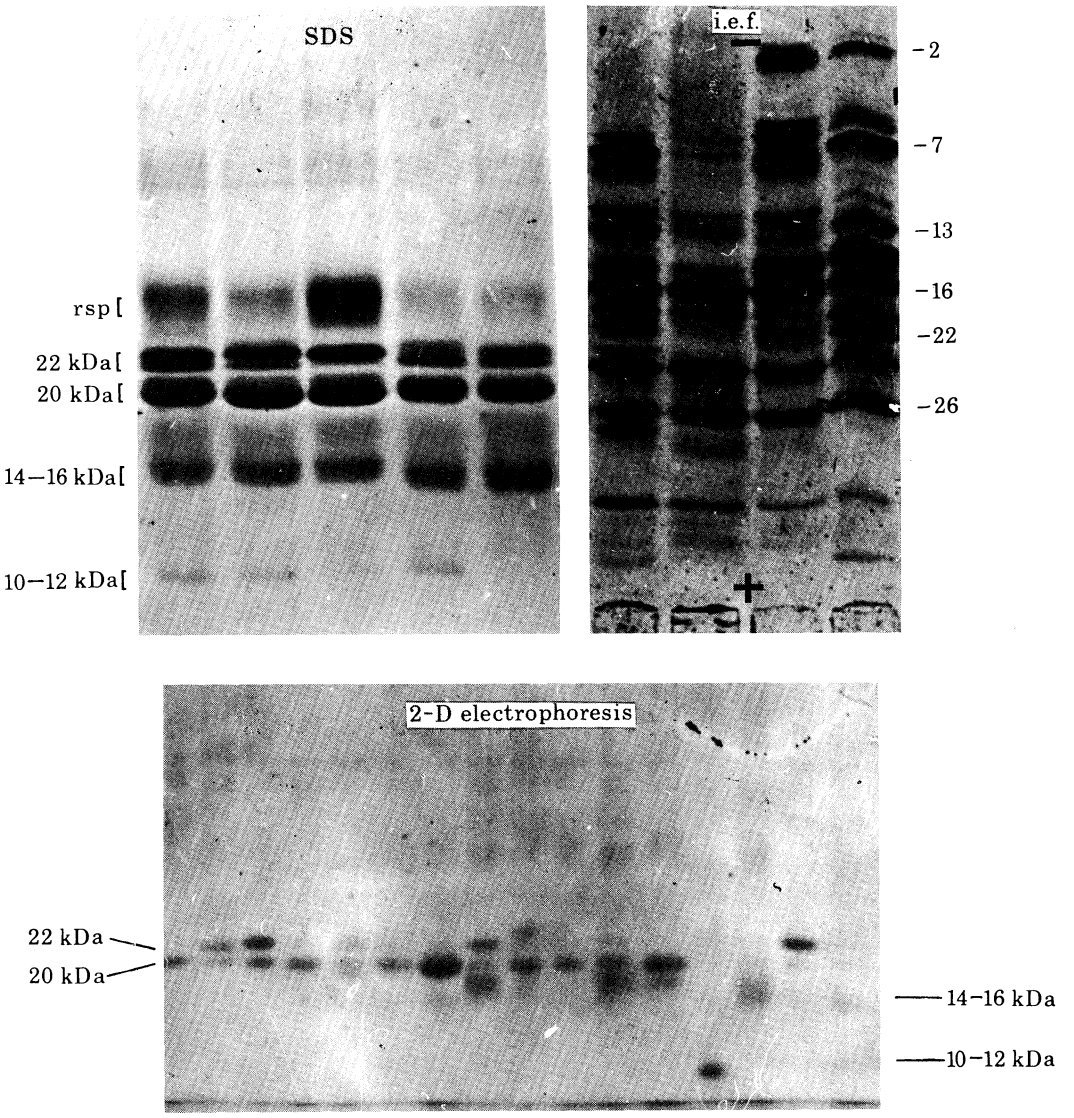

FIGURE 1. Electrophoretic fractionation of zeins. Zeins, extracted from mature endosperms with 70% ethanol (by volume), were fractionated by SDS, i.e.f. and two-dimensional (i.e.f.-SDS) polyacrylamide gel electrophoresis. Four major groups of zeins are present in SDS gels with molecular masses of about 22, 20, 14–16 and 10–12 kDa, and a non-zein protein (rsp) which is soluble in alcoholic solutions. I.e.f. resolves several zein components, denoted by numbers increasing from the more alkaline to the more acidic ones. Different lanes refer to the SDS and i.e.f. patterns of zeins from different inbred lines of maize. Note the quantitative and qualitative variability existing among maize genotypes. For 2-D electrophoresis, individual zein i.e.f. bands were excised and subjected to SDS electrophoresis. Note the presence of more than one polypeptide, differing in M_r in individual zein i.e.f. components.

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in general in each site the individual zein genes are not contiguous, but slightly dispersed. An exception is the *Zp20/1*, *Zp20/2*, *Zp20/3* cluster on chromosome 7 whose members are strictly linked and the corresponding i.e.f. zein components are always expressed or completely silent. On the whole, this arrangement shows some similarity to other dispersed multigene families such as the actin genes in moulds and in *Drosophila* (Kindle & Firtel 1978; Tobin *et al.* 1980).

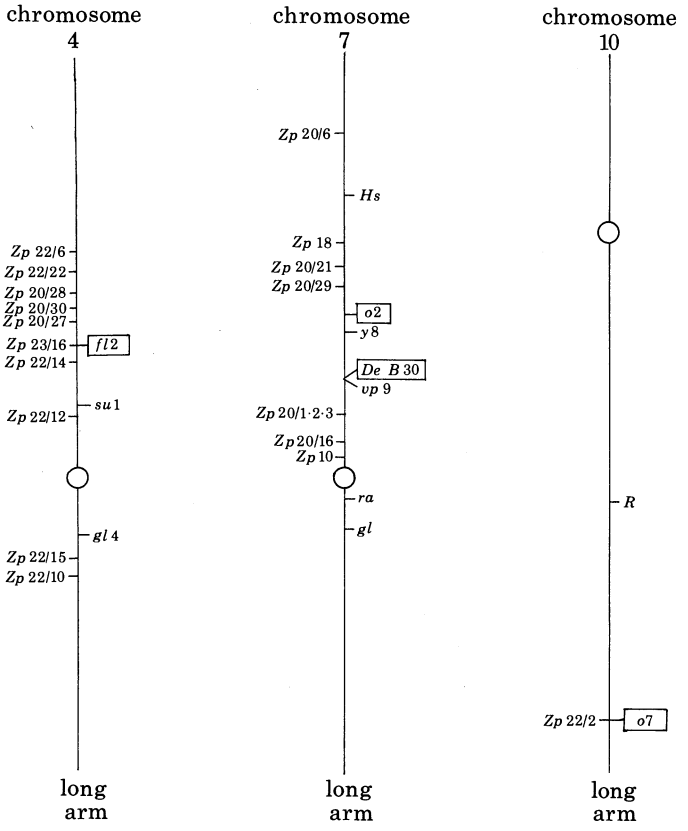

FIGURE 2. Schematic diagram of maize chromosomes 4, 7 and 10, showing the position of some structural zein genes (*Zp*), of zein regulatory loci *Fl2*, *O2*, *O7* and *De-B30* (in boxes) and of other genetic markers of the chromosomes considered. For the nomenclature of zein genes, see the text.

Secondly, in each site genes encoding for zein polypeptides of different M_r coexist. For example on chromosome 4, near the *Floury-2* (*Fl2*) locus, genes encoding for 23, 22 and 20 kDa polypeptides are located, and on chromosome 7, besides a majority of genes encoding for 20 kDa zeins, a gene for a 18 kDa and another for a 10 kDa zein are present. It is interesting that two alleles of the latter gene were found encoding for two polypeptides of slightly different M_r (Bonanomi *et al.* 1983). It could be that the interspersion of zein genes of different M_r in the same chromosomal region is the consequence of a generation of M_r variants caused by the occurrence of nonsense mutations resulting in premature termination of translation (Spena *et al.* 1982). Thirdly, in the same chromosomal regions some loci (*Fl2*, *O2*, *De-B30*, *O7*) controlling the rate of zein accumulation are present and in at least one case they are duplicated (i.e. the *O2* and *De-B30* loci).

Molecular genetics

A reasonable agreement exists between the location of zein genes as detected by formal genetical analysis and by *in situ* hybridization using zein cDNA sequences. By using this technique, sequences related to cDNAs corresponding to 20 kDa zeins were found on the short arm of chromosome 7, on the long arm of chromosome 10 and on the proximal part of the long arm of chromosome 4, while sequences homologous to 22 kDa zeins were located on the distal end of the long arm of chromosome 4 (Viotti *et al.* 1982). Experiments with zein cDNA clones have identified up to now at least five different groups, or families, of zein sequences consisting of two to three more homologous groups for the 22 kDa zeins, two to three related groups for the 20 kDa zeins and one for the 14 kDa zeins (Burr *et al.* 1982; Marks & Larkins 1982; Geraghty *et al.* 1982; Viotti *et al.* 1982). Generally the extent of homology is higher within members of the same group or among groups belonging to the same M_r class although sequences homologous to members of one M_r class but encoding polypeptides of the other M_r class are present. Possibly this is because of variations in the position of the terminator codon.

On the whole, however, it is evident that all the zein sequences so far described have a reasonable degree of homology, based on similarities between the signal peptide region, on the periodic structure of the 20 amino acid repetitive blocks and on the tail piece. The homology suggests that zein genes originated by duplication and subsequent divergence of an ancestral gene, itself arising by duplication from a gene containing only one repeat unit (Spena *et al.* 1982). As far as the spatial arrangement of zein genes along the DNA molecule is concerned, either dispersed or clustered zein genes exist. In one case for example a genomic clone, about 15 kilobases long, contains four zein genes encoding for polypeptides of 20 kDa (N. Di Fonzo & B. Larkins, personal communication).

Regulation of zein synthesis

Maize endosperm follows a defined sequence of events during development. From fertilization to 13–15 d after pollination endosperm cells divide intensively. Then, around 15 d after pollination, the deposition of storage products (starch and zeins) begins and continues until 45 d after pollination when the formation of a black layer at the base of the seed prevents further uptake of nutrients from the maternal tissue into the endosperm. From this stage the seed progressively dehydrates. Zein synthesis starts around 15 d after pollination and afterwards zeins are accumulated at a constant rate (about 300 $\mu g\ d^{-1}$ per endosperm) for more than 20 d. A particular feature is that all the individual components of the zein family are simultaneously and synchronously synthesized. At least two kinds of control, in principle, could be envisaged: one programming temporally the onset of zein synthesis and the second controlling the rate and the synchronization of zein deposition.

Temporal control

Developmental mutants altering the timing of zein synthesis have been reported (Manzocchi *et al.* 1980). These exhibit a more or less defective endosperm and have a lower than normal zein content at maturity. On the basis of the timing of appearance of the zein components they can be grouped into two classes: those delaying the onset of the deposition of all the zein components and those delaying only the synthesis of some zein components. For example the

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three recessive mutants *de-B6*, *de-B18* and *de-B22* delay the onset of deposition of the 22 kDa zein class for at least 10 d. It is interesting that *de-B18* shows, in addition, very low levels of auxins in the endosperm during development (our unpublished observations). Unfortunately at present no detailed biochemical data are available for these mutants.

TABLE 1. PROPERTIES OF MUTANT ALLELES AT LOCI CONTROLLING ZEIN DEPOSITION

locus	chromosomal location	inheritance	zein inhibition, %	specificity on zein subunits	other properties
Opaque-6 (*O6*)	unknown	recessive	88.5	aspecific	absence of b-32 protein
Opaque-7 (*O7*)	chromosome 10 long arm	recessive	77.5	mainly 22 kDa subunits	—
Opaque-2 (*O2*)	chromosome 7 short arm	recessive	47.0	mainly 20 kDa subunits	absence of b-32 protein high RNAase level
Floury-2 (*Fl2*)	chromosome 4 short arm	semidominant	34.6	aspecific	increased level of b-70 protein altered protein bodies morphology
Mucronate (*Mc*)	unknown	dominant	29.0	aspecific	increased level of b-70 protein
Defective endosperm-B30 (*De-B30*)	chromosome 7 short arm	dominant	12.0	only 22 kDa subunits	increased level of b-70 protein

Rate control

Several loci control positively the rate of zein deposition during endosperm development. The mutant alleles at these loci reduce the zein level to a different extent and affect the deposition of all the zein polypeptides or of some groups preferentially (Soave & Salamini 1983). All mutants confer an opaque phenotype to the endosperm. Table 1 summarizes the available data related to the mutants so far studied. As far as the level of zein polypeptides is concerned, the *O2* and *De-B30* mutants preferentially reduce the level of the 22 kDa zeins, *O7* preferentially reduces the 20 kDa zeins, while *Fl2*, *Mc* and *O6* suppress the synthesis of all zeins at the same extent. The lack of production of the zein polypeptides is reflected by a lower zein mRNA level in the mRNA population of *O2*, *O7* and *Fl2* endosperms and at least a lower level of translatable zein mRNAs in *O6*, *Mc*, *De-B30* and in the double mutant *O2Mc* since cDNA–mRNA hybridization data are not available for these latter mutants (Langridge *et al.* 1982*b*; Burr & Burr 1982; Salamini *et al.* 1983). The interactions between some of these mutant alleles have been investigated and the effects on zein production determined in double mutants with pairs of alleles in all possible combinations; *O2* and *O7* were epistatic to *Fl2* but the action of *O2* was independent of *O7* or *Mc* (Di Fonzo *et al.* 1979; Fornasari *et al.* 1982; Salamini *et al.* 1983). These results suggest that in zein synthesis multiple regulatory pathways are active; at least one is related to the 22 kDa zein class and the other to the 20 kDa zein class with *O2* and *O7* involved in the first and second pathways respectively. If the *Mc* action is found to be independent of *O7*, a third pathway involving *Mc* should be expected.

At the molecular level, the mechanism of action of the loci controlling the rate of zein

deposition is largely unknown. Whatever it might be, these loci must act by producing diffusable factors which interfere with zein production since they control the expression of several zein structural genes dispersed in the maize genome. On this basis a search for proteins (other than zeins) associated with the zein regulatory loci was undertaken. Up to now two proteins have been identified: one (b-32 protein) related to the *O2* and *O6* loci, the other (b-70 protein) related to the *Fl2*, *Mc* and *De-B30* loci (Soave *et al.* 1981*b*; Galante *et al.* 1983).

b-32 is a protein of M_r 32000 present in wild type endosperms and absent in *O2* and *O6* mutant alleles. Both the mutations are associated with the loss of immunologically cross reacting material and translatable mRNA for the b-32 protein. The protein is located, in a monomeric form, in the cytosol (or perhaps in nuclei) and, apparently, it does not interact with other proteins. Its biological function is still unknown even if the b-32 level appears to be correlated to the zein level. In particular b-32 is devoid of any inhibitory activity against endospermic ribonucleases, an interesting possibility raised by the higher than normal level of ribonuclease activity in *O2* endosperms. By genetical experiments it was demonstrated that b-32 is encoded by the *O6* locus and then a hierarchy between the *O2* and *O6* loci was proposed: *O2* activates the *O6* locus, which, by producing b-32 protein, controls positively the expression of zein genes.

b-70 is a protein that is present in wild type endosperms but overproduced in *Fl2*, *Mc* or *De-B30* mutants. In agreement with the dominant nature of the three mutants, the b-70 level increases progressively with the doses of the mutant allele present. At early stages of endosperm development b-70 is located essentially in the zein protein bodies where it is easily hydrolysable with proteolitic enzymes (suggesting a likely location on the membrane of the protein body rather than inside the granule). Later, b-70 could be revealed also in those parts of the e.r. devoid of zeins and in soluble cytoplasm. On the basis of the data available it is possible that b-70 interferes *specifically* with the zein synthetic-secretory system or that its abnormal deposition on the protein body membrane *aspecifically* alters the process of zein accumulation. The last suggestion may simply imply that b-70 is a type of storage protein different from zeins, repressed in normal endosperm and derepressed in the dominant mutant alleles *Fl2*, *Mc* and *De-B30*.

References

Bonanomi, S., Soave, C. & Salamini, F. 1983 Genes for minor SDS zein components on maize chromosome 4 and 7. *Maydica* (In the press.)

Burr, B. & Burr, F. A. 1976 Zein synthesis in maize endosperm by polyribosomes attached to protein bodies. *Proc. natn. Acad. Sci. U.S.A.* **73**, 515–519.

Burr, F. A. & Burr, B. 1981 *In vitro* uptake and processing of prezein and other maize preproteins by maize membranes. *J. Cell Biol.* **90**, 427–434.

Burr, B., Burr, F. A., St. John, T. P., Thomas, M. & Davis, R. D. 1982 Zein storage protein gene family of maize. *J. molec. Biol.* **154**, 33–49.

Burr, F. A. & Burr, B. 1982 Three mutations in *Zea mays* affecting zein accumulation. *J. Cell Biol.* **94**, 201–206.

Di Fonzo, N., Fornasari, E., Salamini, F., Reggiani, R. & Soave, C. 1979 Interaction of the mutants *floury-2*, *opaque-7* with *opaque-2* in the synthesis of endosperm proteins. *J. Hered.* **71**, 397–402.

Fornasari, E., Di Fonzo, N., Salamini, F., Reggiani, R. & Soave, C. 1982 *Floury-2* and *opaque-7* interaction in the synthesis of zein polypeptides. *Maydica* **27**, 185–189.

Galante, E., Vitale, A., Manzocchi, L., Soave, C. & Salamini, F. 1983 Genetic control of a membrane component and zein deposition in maize endosperm. *Molec. gen. Genet.* (In the press.)

Geraghty, D. E., Messing, J. & Rubinstein, I. 1982 Sequence analysis and comparison of cDNAs of the zein multigene family. *E.M.B.O. J.* **1**, 1329–1335.

Gianazza, E., Viglienghi, V., Righetti, P. G., Salamini, F. & Soave, C. 1977 Amino acid composition of zein molecular component. *Phytochemistry* **16**, 315–317.

Hagen, G. & Rubenstein, I. 1980 Two-dimensional gel analysis of the zein proteins in maize. *Pl. Sci. Lett.* **19**, 217–223.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Hagen, G. & Rubenstein, I. 1981 Complex organization of zein genes in maize. *Gene* **13**, 239–249.
Kindle, K. L. & Firtel, R. A. 1978 Identification and analysis of *Dictyostelium* actin genes, a family of moderately repeated genes. *Cell* **15**, 763–778.
Langridge, P., Pintor-Toro, J. A. & Feix, A. 1982*a* Zein precursors mRNA from maize endosperm. *Molec. gen. Genet.* **187**, 432–438.
Langridge, P., Pintor-Toro, J. A. & Feix, G. 1982*b* Transcriptional effects of the *opaque-2* mutation of *Zea mays*. *Planta* **156**, 166–170.
Larkins, B. A. & Hurkman, W. I. 1978 Synthesis and deposition of zein in protein bodies of maize endosperm. *Pl. Physiol.* **62**, 256–263.
Lee, K. H., Jones, R. A., Dalby, A. & Tsai, C. Y. 1976 Genetic regulation of storage protein content in maize endosperm. *Biochem. Genet.* **14**, 641–650.
Manzocchi, L. A., Daminati, M. G. & Gentinetta, E. 1980 Viable defective endosperm mutants in maize. *Maydica* **25**, 199–210.
Marks, M. D. & Larkins, B. A. 1982 Analysis of sequence microheterogeneity among zein messenger RNAs. *J. biol. Chem.* **257**, 9976–9983.
Melcher, U. 1979 *In vitro* synthesis of a precursor to the methionine-rich polypeptide of the zein fraction of corn. *Pl. Physiol.* **63**, 354–358.
Pedersen, K., Devereux, J., Wilson, D. R., Sheldon, E. & Larkins, B. A. 1982 Cloning and sequence analysis reveals structural variation among related zein genes in maize. *Cell* **29**, 1015–1026.
Righetti, P. G., Gianazza, E., Viotti, A. & Soave, C. 1977 Heterogeneity of storage proteins in maize. *Planta* **136**, 115–123.
Salamini, F., Di Fonzo, N., Fornasari, E., Gentinetta, E., Reggiani, R. & Soave, C. 1983 Mucronate, *Mc*, a dominant gene of maize which interacts with *opaque-2* to suppress zein synthesis. *Theoret. appl. Genet.* (In the press.)
Soave, C., Reggiani, R., Di Fonzo, N. & Salamini, F. 1981*a* Clustering of genes for 20 k zein subunits in the short arm of maize chromosome 7. *Genetics* **97**, 363–377.
Soave, C., Reggiani, R., Di Fonzo, N. & Salamini, F. 1982 Genes for zein subunits on maize chromosome 4. *Biochem. Genet.* **11**, 1027–1037.
Soave, C. & Salamini, F. 1982 Zein proteins: a multigene family from maize endosperm. *Qual. Pl. Pl. Fds Hum. Nutr.* **31**, 191–203.
Soave, C. & Salamini, F. 1983 Genetic organization and regulation of maize storage proteins. In *Seed proteins* (ed. J. Daussant, J. Mossé & J. Vaughan), pp. 205–218. New York and London: Academic Press.
Soave, C., Tardani, I., Di Fonzo, N. & Salamini, F. 1981*b* Regulation of zein level in maize endosperm by a protein under control of the *opaque-2* and *opaque-6* loci. *Cell* **27**, 403–410.
Spena, A., Viotti, A. & Pirrotta, V. 1982 A homologous repetitive bloch structure underlies the heterogeneity of heavy and light chain zein genes. *E.M.B.O. J.* **1**, 1589–1594.
Tobin S. L., Zulauf, E., Sanchez, F., Craig, E. A. & McCarthy, B. J. 1980 Multiple actin-related sequences in the *Drosophila melanogaster* genome. *Cell* **19**, 121–131.
Valentini, G., Soave, C. & Ottaviano, E. 1979 Chromosomal location of zein genes in *Zea mays*. *Heredity* **42**, 33–40.
Viotti, A., Sala, E., Marotta, E., Alberi, P., Balducci, C. & Soave, C. 1979 Genes and mRNA coding for zein polypeptides in *Zea*. *Eur. J. Biochem.* **102**, 211–222.
Viotti, A., Abildsten, D., Pogna, N., Sala, E. & Pirrotta, V. 1982 Multiplicity and diversity of cloned zein cDNA sequences and their chromosomal localisation. *E.M.B.O. J.* **1**, 53–58.
Vitale, A., Soave, C. & Galante, E. 1980 Peptide mapping of IEF zein components from maize. *Pl. Sci. Lett.* **18**, 57–64.
Wienand, U. & Feix, G. 1980 Zein specific restriction enzyme fragments of maize DNA. *FEBS Lett.* **116**, 14–16.
Wienand, U., Langridge, P. & Feix, G. 1981 Isolation and characterization of a genomic sequence of maize coding for a zein gene. *Molec. gen. Genet.* **182**, 440–444.

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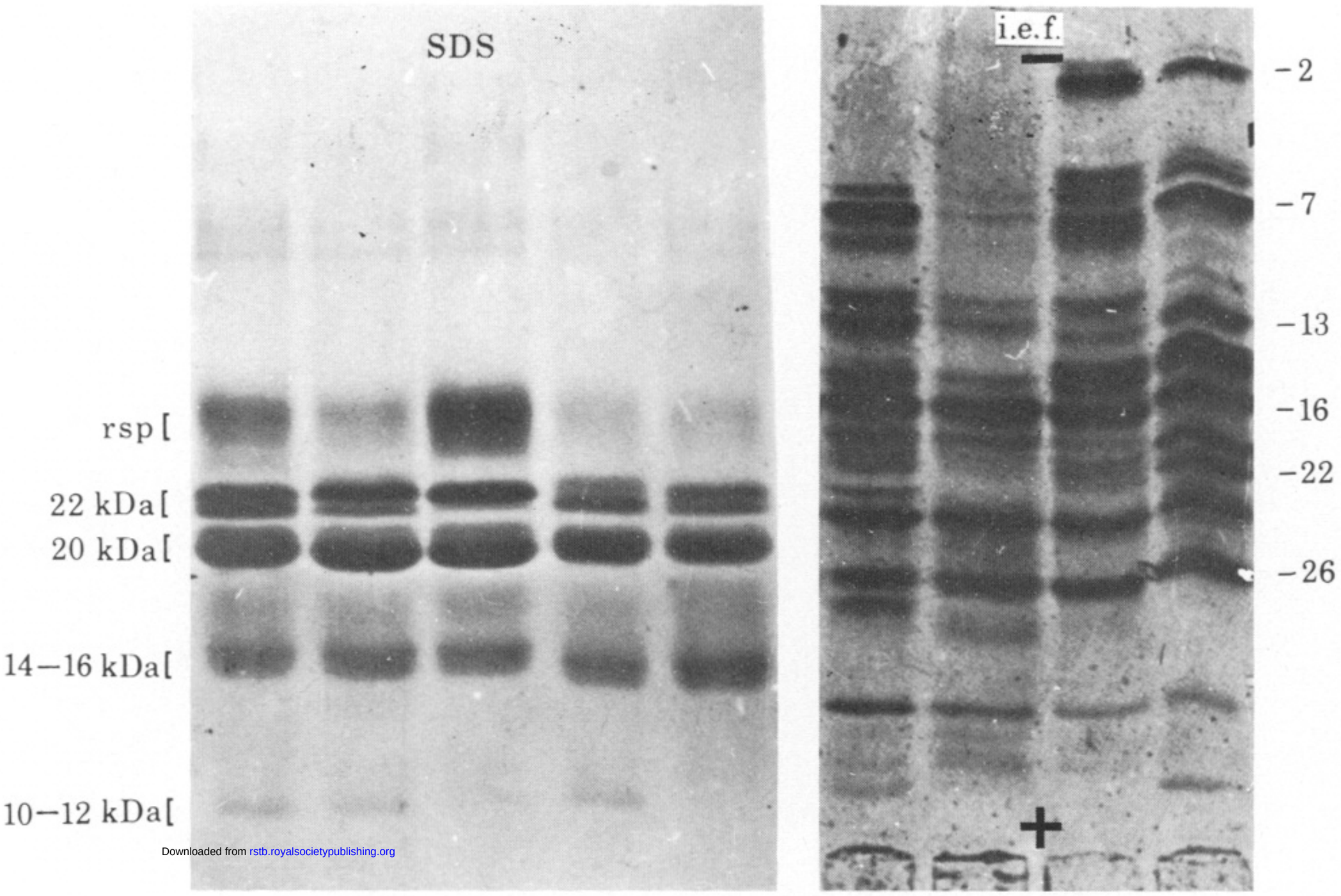

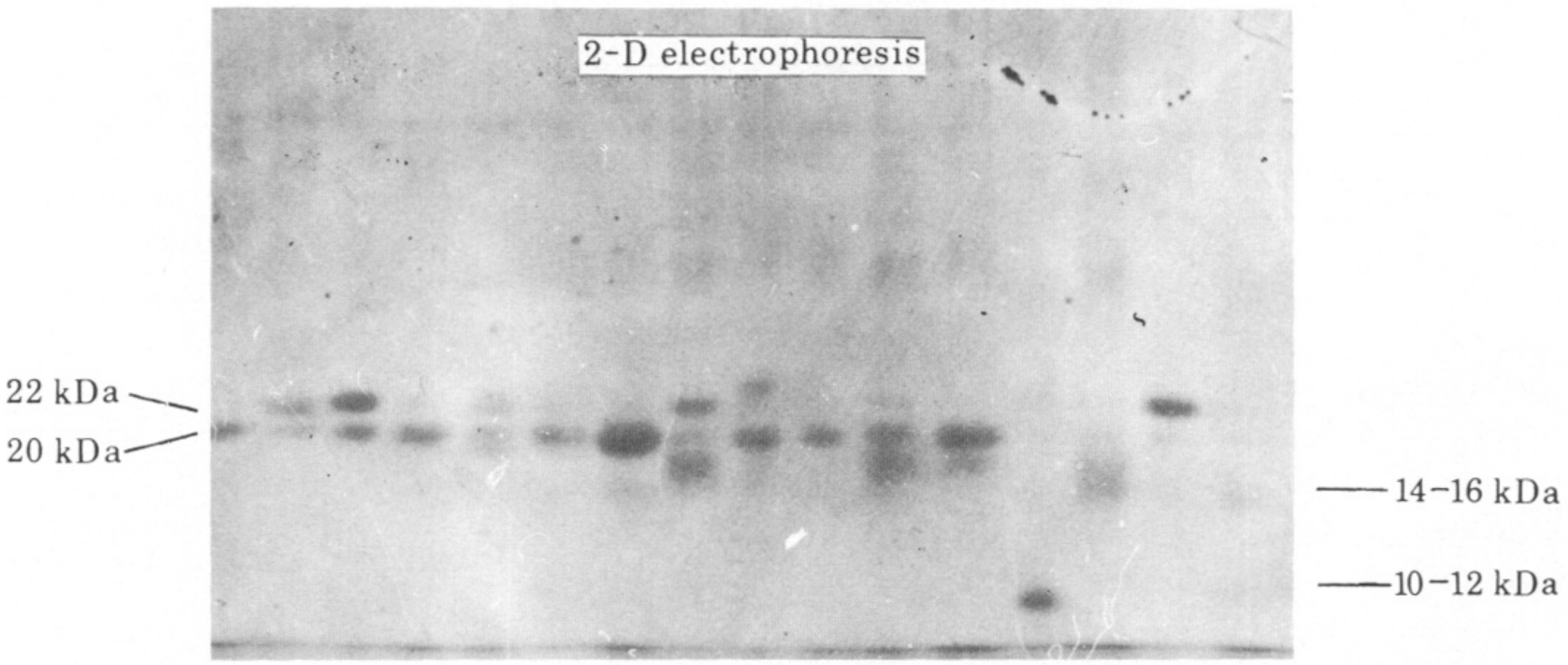

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