Dordrecht Group, Dordrecht/Boston/Lancaster.
Warncke, K., & Dutton, P. L. (1990) in *Current Research in Photosynthesis* (Baltscheffsky, M., Ed.) Vol. 1, pp 157–160, Kluwer Academic Publishers, Dordrecht/Boston/London.
Woodbury, N. W., Parson, W. W., Gunner, M. R., Prince, R. C., & Dutton, P. L. (1986) *Biochim. Biophys. Acta 851*, 6–22.

A Shared Internal Threonine–Glutamic Acid–Threonine–Proline Repeat Defines a Family of *Dictyostelium discoideum* Spore Germination Specific Proteins†

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ABSTRACT: A cDNA denoted pRK270 hybridizes to two mRNA species in RNA blots. The mRNAs specific to this clone are not expressed during vegetative growth and multicellular development. They are, however, found predominantly during early stages of spore germination, suggesting that their synthesis is rapidly and coordinately turned on during germination. Two different cDNAs named 270-6 and 270-11 were isolated, representing the two mRNAs. DNA blot analysis shows that 270 is a multigene family. Four genes were isolated from *Dictyostelium* genomic libraries and sequenced. The putative proteins coded for by these genes are about 51 000, 55 000, 76 000, and 100 000 Da. Two of the genes are expressed during spore germination while transcripts for the other two are not present during spore germination, vegetative growth, or the stages of multicellular development studied. The cDNAs and genes code for deduced proteins that possess a very unusual internal amino acid repeat comprised of the tetrapeptide threonine–glutamic acid–threonine–proline. The other portions of the proteins have no homology among themselves. The 270-6 protein shows excellent identity with avocado (*Persea americana*) cellulase, indicating that it may function as an *endo*-(1,4)-β-D-glucanase.

Dictyostelium discoideum is a favorable organism for studying the macromolecular events coincident with and necessary for eukaryotic development. The life cycle is short, and development can be synchronized and separated from vegetative growth (Sussman & Brackenbury, 1976). Consequently, biochemical and morphological changes that occur can be correlated, and developmentally critical events may be revealed. In addition, the organism has a small genome, which makes its analysis and selection of specific genes easier than in more complex eukaryotes.

One of the central problems of developmental biology is to determine the nature of the mechanisms that control the activation and expression of developmentally critical genes. For this purpose it is first necessary to isolate and identify these genes. In previously reported work we identified proteins that are developmentally regulated during spore germination (Dowbenko & Ennis, 1980; Giorda & Ennis, 1987; Giri & Ennis, 1978; Kelly et al., 1983). Spore germination in *D. discoideum*, similar to other stages in slime mold development, is accompanied by developmentally regulated changes in both protein and mRNA synthesis (Dowbenko & Ennis, 1980; Giri & Ennis, 1978; Kelly et al., 1983), and this makes the process a favorable one for developmental studies. A number of cDNA clones were isolated representing mRNA that is present only in spores and/or during spore germination, and these cDNAs have been used to isolate specific genomic sequences. One developmentally regulated cDNA on which the present study focuses, named pRK270, hybridized to mRNA present almost exclusively during early spore germination. This mRNA did not accumulate during growth or multicellular development and was present in very low concentration in dormant spores (Kelly et al., 1983; Shaw et al., 1986). pRK270 is a member of a multigene family containing four different genes, and we have isolated and sequenced all of them. A common feature of the deduced protein sequences is an internally located repeat of the tetrapeptide threonine–glutamic acid–threonine–proline.

Experimental Procedures

Previously Described Methods. All methods involving growth of *D. discoideum*, preparation of spores and analysis of spore germination, isolation of RNA and DNA, RNA and DNA blot analysis, labeling of DNA probes, DNA sequencing, and sources of materials were those described (Giorda & Ennis, 1987).

Preparation of cDNA and Genomic Libraries. cDNA libraries were prepared from poly(A)$^+$-selected 1.5-h germination RNA. The libraries were constructed in λgt10 as described by the manufacturer of the cloning kit (Amersham, Arlington Heights, IL). The two cDNAs isolated were named λTO270-6 (which contains the sequences of the original pRK270 clone but is longer) and λTO270-11.

A genomic library of sheared AX3 DNA, to which were added *Eco*RI linkers, was cloned in λZAP bacteriophage by Strategene (San Diego, CA). Most clones were isolated from this library. Clone 270-P was isolated from another library constructed by using approximately 6-kb *Eco*RI fragments inserted into λgt10. One small clone containing the 270-11 intron (270-PCR) was constructed by using the polymerase

† The nucleic acid sequences in this paper have been submitted to GenBank under Accession Number J02916.
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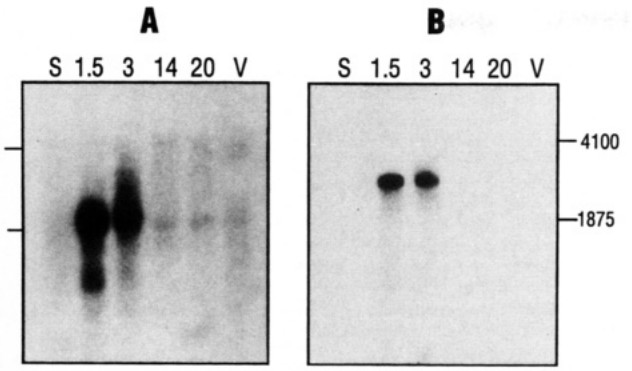


FIGURE 1: Developmental expression of 270-specific mRNA during *D. discoideum* spore germination and multicellular development. Total RNA (10 μg/lane) isolated from dormant spores (S), spores at 1.5 h (1.5) and 3 h (3) of germination, vegetative cells (V), and cells at 14 h (14) and 20 h (20) of multicellular development were fractionated on a 1.5% agarose–6% formaldehyde gel, transferred to Gene Screen, and hybridized to nick-translated plasmid probe. The filter was washed and exposed to X-ray film. The markers are *D. discoideum* ribosomal RNAs. Panel A: a 540-bp fragment of 270-11 (probe 2 in Figure 2) was used as probe. Panel B: the probe was a 340-bp fragment of 270-6 (probe 1 in Figure 2). The sizes of ribosomal RNA markers are indicated.

chain reaction (White et al., 1989). The notation given for the various clones is presented in Figure 2.

Positive clones were subcloned either in M13mp18 (Yanisch-Perron et al., 1985) or Bluescript (Stratagene). Sequential deletions to allow complete sequencing of each clone were obtained by *Exo*III/S1 nuclease treatment (Henikoff, 1984), preferably in both directions. Single-stranded DNA was extracted from M13 as described (Giorda & Ennis, 1987) and from Bluescript by superinfection with R408 helper phage according to the manufacturer's instructions.

The sequences obtained were stored and processed by using the DNASTAR program (DNASTAR, Inc., Madison, WI) and the Genetics Computer Group sequence analysis software package version 6.0 (Devereux et al., 1984).

Results and Discussion

In higher eukaryotes' short discrete sequences have been shown to be necessary for both temporal and spatial transcription to occur (Jaynes & O'Farrell, 1988; Landschultz et al., 1989; Turner & Tjian, 1989). The interaction of trans-acting cellular protein factors with cis-acting DNA sequences regulates transcription of differentially expressed genes (Jaynes & O'Farrell, 1988; Landschultz et al., 1989; Turner & Tjian, 1989). In order to understand gene regulation in *Dictyostelium* it is therefore necessary to identify all elements essential for temporal and spatial inducibility of regulated genes.

Because we are interested in understanding gene structure as it relates to developmental regulation, we initiated a study to identify such regulated genes in the cellular slime mold. The fact that the organism has a small genome makes it a highly favorable one for the analysis and selection of particular genes. A previously isolated cDNA clone, denoted pRK270 (Kelly et al., 1983), hybridized to two mRNA species specific for spore germination, the larger one about 2900 nt and the smaller one 2000 nt. By use of pRK270 as a probe, two different full-length cDNAs were isolated from a λgt10 1.5-h germination library. λTO270-11 (Figure 1, panel A), which represents the 2000-nt mRNA species, and λTO270-6 (Figure 1, panel B), which is the 2900-nt species, seem to be regulated coordinately. Although a large number of 270-specific cDNA clones were screened, no other different cDNAs were identified. RNAs specific to the two clones were not found during vegetative growth and multicellular development. The mRNAs were present at very low concentration in dormant spores and their concentrations increased rapidly during the early stages of spore germination, indicating that 270-specific mRNA synthesis might be rapidly turned on during germination. The RNAs accumulated to a peak concentration after

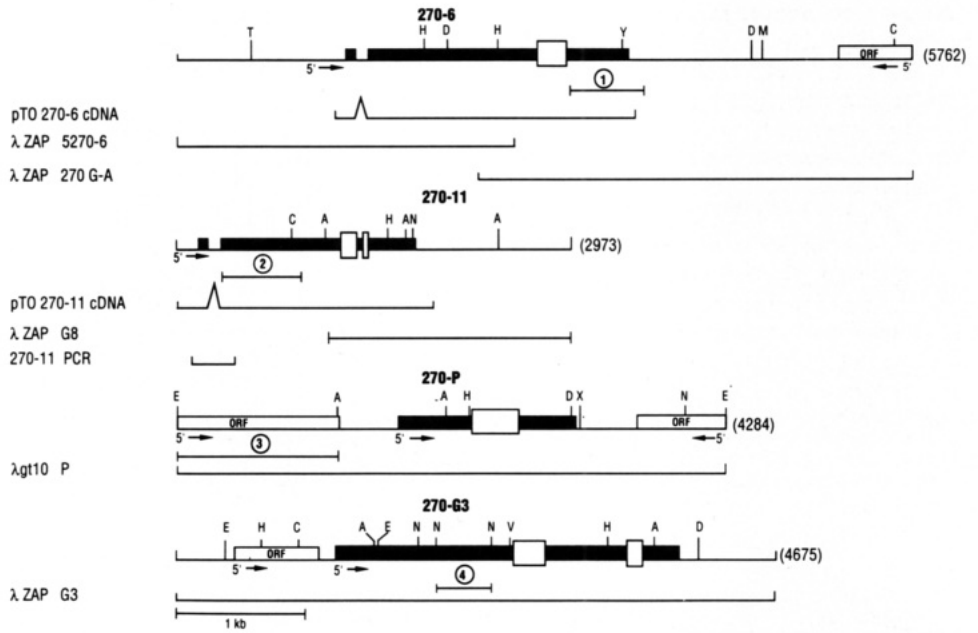
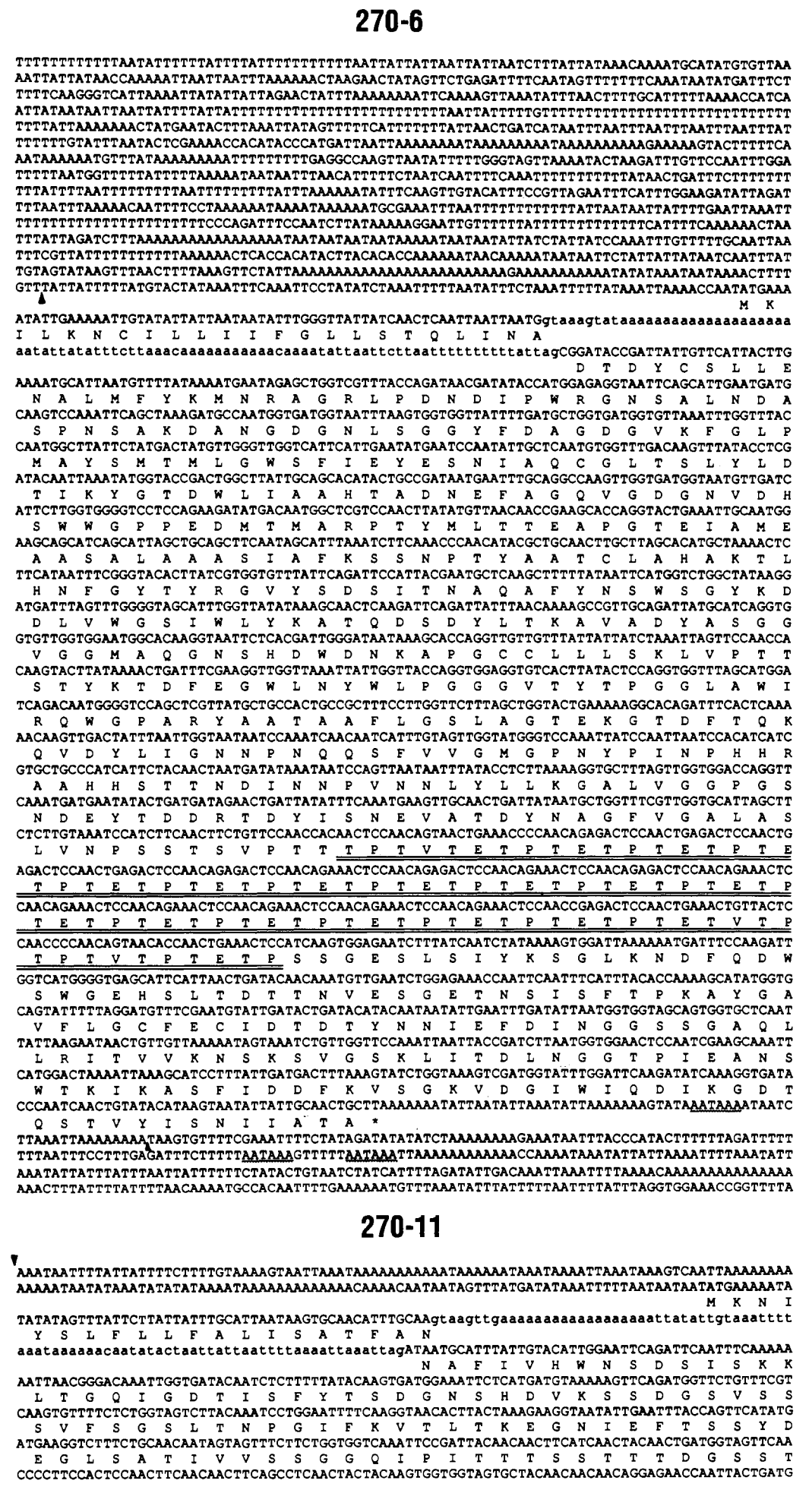


FIGURE 2: Genomic organization and restriction map of 270 genes. The DNA fragments shown are the entire extents of the genes and their surrounding regions that have been isolated and sequenced. The closed boxes are the open reading frames deduced from the sequence data. The open boxes within these regions are the repeats (see Figures 3 and 5). The interruptions in the closed boxes at the 5′-end of 270-6 and 270-11 indicate the introns. The open boxes denoted ORF represent open reading frames deduced from the sequence. The orientation of the open reading frames is indicated by the arrow and shows the sense direction. Regions of the DNA fragments used as probes for RNA (Figure 1) and DNA blot (Figure 4) are denoted by the lines marked 1–4 under each sequence. The length and location of the cDNA and genomic clones are presented as lines below the gene map. The numbers in parentheses are the lengths in nucleotides of the genomic fragments. The letters are abbreviations for restriction enzymes: A, *Ava*II; C, *Cla*I; D, *Hin*dIII; E, *Eco*RI; H, *Hin*cII; M, *Bam*HI; N, *Xmn*I; T, *Hae*III; V, *Eco*RV; and X, *Xba*I.


270-6
270-11

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      P  S  T  P  T  S  T  T  S  A  S  T  T  T  S  G  G  S  A  T  T  T  T  G  E  P  I  T  D  G  155
551   GTTCTAATGGAGGCGCCAGTTCCACAACTGGCAATAGCGGGACGACAGGTTCTGCTACCACTACTACTTCTTCTTCTTCCGATAATTCCG
      S  N  G  G  A  S  S  T  T  G  N  S  G  T  T  G  S  A  T  T  T  T  S  S  S  S  D  N  S  D  185
641   ATGGCAGTGTAGGTACTTCAACTACAACTTCACCAGCTATCACAACTTCAAGTGGGTCAATAATCGATCCAACTTCACCACCTACAACTG
      G  S  V  G  T  S  T  T  T  S  P  A  I  T  T  S  S  G  S  I  I  D  P  T  S  P  P  T  T  D  215
731   ATTCATCCTCTAATAGTGGTGGTTATGGTTCATCATCTTCAATTGAAAATGGCGTAGAATGTTTATTAACAATCACTCAAGATGCATTTG
      S  S  S  N  S  G  G  Y  G  S  S  S  S  I  E  N  G  V  E  C  L  L  T  I  T  Q  D  A  F  D  245
821   ATTCTTGGACATATGATAATATTATTTACACCGTTTATCAAGTAAATTTAACAAATATTGGTACACTTTCAGTTGAGTCTGTTATTCTCA
      S  W  T  Y  D  N  I  I  Y  T  V  Y  Q  V  N  L  T  N  I  G  T  L  S  V  E  S  V  I  L  T  275
911   CTCCAAATGATAACTCTTTAATTTACCATACTTGGGAATTGGTTTATGATGGAACTTCACTCACTCTTCCAACCTATAGAAAAGCTGGTC
      P  N  D  N  S  L  I  Y  H  T  W  E  L  V  Y  D  G  T  S  L  T  L  P  T  Y  R  K  A  G  P  305
1001  CAATCAATCCAGAGGAAACCATTATCTTTGGTTATATCTCTAGAAATAGTACTGATGTTACATTTGCTTTAAGTCCAACATGTTCAGATT
      I  N  P  E  E  T  I  I  F  G  Y  I  S  R  N  S  T  D  V  T  F  A  L  S  P  T  C  S  D  S  335
1091  CATCAAGTCCAACTCCAACTCCTACTGAGACTCCAACTGAGACTCCAACTGAGACTCCAACTGAGACTCCAACTGAGACTCCAACTGAAA
      S  S  P  T  P  T  P  T  E  T  P  T  E  T  P  T  E  T  P  T  E  T  P  T  E  T  P  T  E  T  365
1181  CTCCAACTGAAACTCCAACTGAAACTGAAACTCCAACACCAACACCATCAAGCTCATCTAGTGATGTAGATAGTGGTTCATCATCTGAAA
      P  T  E  T  P  T  E  T  E  T  P  T  P  T  P  S  S  S  S  S  D  V  D  S  G  S  S  S  E  I  395
1271  TTGAAACCCCAACACCAACTGAAACTGATACCCCAACCCCAACACCATCAAGTTCTTCAAGTGAAGGAAGTGGATCATCATCAGAAACTC
      E  T  P  T  P  T  E  T  D  T  P  T  P  T  P  S  S  S  S  S  S  E  G  S  G  S  S  S  E  T  Q  425
1361  AACCACCAATTACTCCACCACCAACCACTGGTACTTCTTGTTTAGCCCAAGTCCAACAAAAAGTTATCAACTCATGGATTAATGGTGAAG
      P  P  I  T  P  P  P  T  T  G  T  S  C  L  A  Q  V  Q  Q  K  V  I  N  S  W  I  N  G  E  V  455
1451  TTGATCATTATATACAAGTTGAGGCTACTATTGTTAACCAAGGTTCAACTCCAATTTCATCTTTTAATTTTTATTCTGATGCTGAACAAA
      D  H  Y  I  Q  V  E  A  T  I  V  N  Q  G  S  T  P  I  S  S  F  N  F  Y  S  D  A  E  Q  I  485
1541  TTTGGTCAGTTGAAAAAACAGGAACCAATACCTATAAATTACCAAGTTGGTTCTCAACAATTCCAGTTGGTGGGTCCCATACCTTTGGTT
      W  S  V  E  K  T  G  T  N  T  Y  K  L  P  S  W  F  S  T  I  P  V  G  G  S  H  T  F  G  Y  515
1631  ATATTGTTAAATCTGCTGAATTATCTGACCTCGAAGGAGTTCAATATACATGTTGATTTTAAAACTCTCTTTTTGTAATAATAAAAAAAA
      I  V  K  S  A  E  L  S  D  L  E  G  V  Q  Y  T  C  *                                    532
1721  AAAAAAAATTTTTTGGAAATAAATTTAATTTTCAAAAACTAGTTTTGATTTCACTTTATTTAATAATAAAAATAATTTTGAATTAAAAAA
1811  ACACAATAAAGGAAATAAATAAATAAATTTGTAACATTTAAAAATAATACCGTTATTAATTATTTTTAATTTTTAATTTTTACTTTTTTT
1901  TAATGTTGGATTTTAAAGTTAAAAGCCTAAAAAAAAAAAATAATAATTTCCTTCCTCTTTTTTATTTAATTCCTTTTTTTTTTTATTTTTTT
1991  TAGCCTCTTTTTTTTTTTTTTAATTTTTTTTTTTTTCTTTAAAAATTTATAATATATAATTCCTTATTTAACACTTTTTTTATTTTGTTC
2081  CTTTTTATTTTAATAATTTATAAATAAGTTTTTATTATTTTTAAAAAAATTGAAAAATTAGTTTTCTAAAATTTTTTTGCAACTCAAGTT
2171  TGAATCTTTAAAATATCTAAATGTCCTATGATAATAAATAACTTATTTTATAATCCAGGCTTAAACCTGGTAACTTCGAAGTTTAAAAAA
2261  AAAAAAAAAAAACAAATATAAAAACAAAAAAAAAAAAAAAAAATTAAATTTCAATTTTCAGTTGGACCAGGTTTTTTTTTTTTTAATTTTT
2351  TTAAATTAAATAATAAGATTTATTTTTATTTTTAATTAAATTTAATTTTTAAAATTTATATATAAATGTTTTTATTTTTATTTTTTTTAT
2441  AGGGATTAAAAAATAAAGTAGTAAAAAAGTAATAAATAAATTAATAAAATAAAAAATAATAAATTAATAAAATAAAATAAAATAAAATAT
2531  TAAATTAAAACAAAATAAATTAAATGAACACTAATATTAATACATATAACCAAAATATAAATAGTGGCGAAAGTGATGTAATTTTAAATA
2621  TTCCATTATTATTAAACAAATCACAAAGAAATATAAATGTTAATTATGGTACAATTTCACAATCAGTACCACCACTACCATCACAACCAC
2711  TCTCAAAAACAAATCATCAACCAACTTCACAAGTCTATCAAATCCAAGCACAAAATCAACAACAACAACAACAACAACAATTAAATAATG
2801  TATTGGTATTAAATAAAAGTAAAAAAACATTATTGTTTGATAATAATGTTTATTATGGTGAATTAACGCCATACATAAATCCACAATAAT
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FIGURE 3: Nucleotide and amino acid sequences of 270-6 and 270-11 genes. The sequence of 270-6 presented in this figure includes nucleotide numbered 1201–3870 in the map presented in Figure 2. The sequence of 270-11 is from nucleotide 1 to 3060 (which is the complete fragment shown in Figure 2). They represent an arbitrary selection of some of the 5′-upstream and 3′-downstream sequences, but all of the deduced coding regions. Position +1 in the figure is defined as the first base of the putative initiation codon of the 270 protein. The nucleotide number is given on the left and the amino acid residue number on the right. The repeat regions are double underlined. The arrows indicate the extent of cDNAs λTO270-6 and λTO270-11. The introns are shown in lower case letters. Possible poly(A) adenylation sites are underlined with a wavy line. Amino acids are denoted in the single letter code underneath the nucleotide sequence.

1–2 h during germination and their levels declined thereafter. These results showed that 270 mRNA was developmentally regulated during *Dictyostelium* development.

DNA blot analysis of *Dictyostelium* genomic DNA indicated that the 270 genes constituted a multigene family comprised of probably four or five different genomic fragments (data not shown). Consequently the genes representing the 270 family were isolated from various genomic libraries and sequenced and their genomic organization established. Four genomic fragments were isolated (Figure 2). From sequence data (Figure 3), it was possible to establish that genomic fragments 270-6 and 270-11 represented cDNAs λTO270-6 and -11, respectively.

The location of each of the genomic clones on individual restriction fragments of genomic DNA was determined. The respective probes indicated 1–4 in Figure 2 were used to screen DNA blots of genomic DNA cleaved with the indicated restriction enzymes. The results presented in Figure 4 show that each of the genomic clones was represented by a different single copy sequence in the *Dictyostelium* genome.

If each gene represents a unique sequence, what is their common feature that allows cross hybridization among them all? It is obvious from the sequences presented in Figure 3 that the common feature is a sequence that codes for an unusual internal amino acid repeat comprised, in the most part, of the tetrapeptide threonine–glutamic acid–threonine–proline. The relevant repeats among the deduced proteins are presented in Figure 5. 270-6 and 270-P had single long repeats of the tetrapeptide, while 270-11 and 270-G3 had two of the repeat

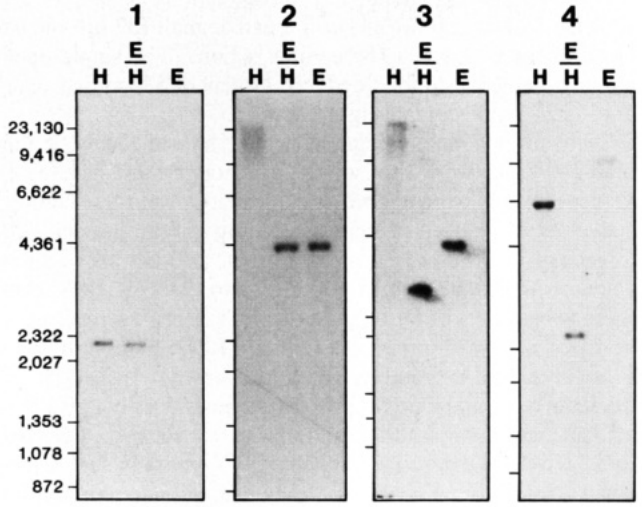

FIGURE 4: Genomic localization of 270 sequences. Five micrograms of nuclear DNA was digested to completion with *Hin*dIII (H), *Hin*dIII plus *Eco*RI (E/H), or *Eco*RI (E), size-fractionated on a 0.8% agarose gel, transferred to nitrocellulose, and hybridized to the indicated nick-translated probe. The washed filter was exposed to X-ray film. The positions of the relevant size markers in bp (λ phage digested with *Hin*dIII and θX174 digested with *Hae*III) are indicated. Panels 1–4 represent results using the respective probes marked 1–4 on the restriction map in Figure 2. Note that none of the probes contained the common repeat but represented unique sequences.

regions separated by a stretch lacking the repeat. The codons CCA for proline, ACU and ACA for threonine, and GAA for glutamate were used overwhelmingly in the repeats. This is

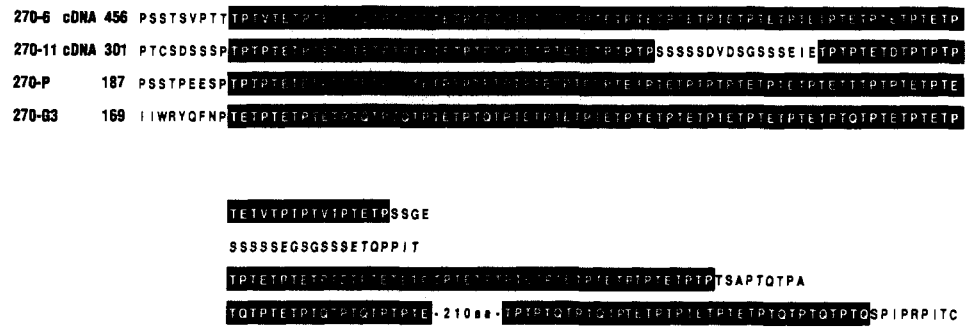

FIGURE 5: Amino acid sequences of the *Dictyostelium* 270 internal repeats. cDNA clones λTO270-6 and λTO270-11 and all genomic clones (see Figure 2) were sequenced. Only the relevant amino acid repeats of the deduced proteins are presented to show the homology, including some flanking amino acids. The sequences shown represent the open boxes contained in the 270 coding regions presented in Figure 2. The number to the left of the sequence is the amino acid number of the respective protein. The amino acids are presented in the single-letter code and the enclosed boxed letters are part of the repeat.

consistent with the normal codon usage for these amino acids in *Dictyostelium* (Warrick & Spudich, 1988).

The portions of the deduced proteins that did not contain the repeat did not have homology among themselves (data not shown) and thus represent distinct proteins that merely share a common repeat.

270-6 coded for a deduced protein containing 705 amino acids with a minimum molecular weight of 75 918. It contained one small intron (Figures 2 and 3). The 1344 bp 5′ to the initiator codon should contain regulatory sequences, and we therefore will be able to study the regulation of this gene's expression in the *Dictyostelium* transformation system using the properly constructed vectors (Cohen et al., 1986; Early & Williams, 1987; Nellen et al., 1984). The gene contained one long repeat of the tetrapeptide. The putative protein is likely to contain a hydrophobic leader sequence.

The 270-11 sequence was incomplete, lacking most of the 5′ nontranscribed portion. Although we have tried numerous ways to clone this region, screening a variety of genomic libraries, we have been uniformly unsuccessful in accomplishing it. Like 270-6, gene 270-11 contained a small (87 bp) intron close to the 5′-start of the coding region. The single open reading frame coded for a deduced protein of 532 amino acids and 55 213 molecular weight.

Two other genomic sequences that hybridized with the pRK270 probe were also isolated. Although these have been sequenced, they were not presented here. One of these, 270-P, coded for a deduced protein containing 460 amino acids of molecular weight 51 267 and the other, 270-G3, 893 amino acids of molecular weight 100 210. No cDNAs specific to these two sequences were found during extensive screening of libraries, nor were transcripts found in RNA blots similar to those presented in Figure 1 (data not shown). Inspection of the sequences of and adjacent to these genes does not give any evident reason why they should not be transcribed. Perhaps these genes are transcribed but the mRNAs are in such a low concentration or so labile that they are undetectable by the method used, or they are transcribed during a time in development that we have not analyzed. We have found similar lack of transcription of "normal" looking genes in the *Dictyostelium* 109 genes (Giorda et al., 1989). It is noteworthy that recently an ostensibly normal mouse major urinary protein gene was found to be silent in the genome of BALB/cByJ mice but expressed when introduced as a 9.4-kb DNA fragment in the mouse germ line. The results suggest that the lack of expression in the genome may be due to an inhibitory position effect, and not to nonfunctional or missing regulatory elements (Shi et al., 1989).

It is noteworthy that unrelated open reading frames were closely linked to three of the genes (Figure 2). This is not unusual in *Dictyostelium* because the genome size is small (Driscoll & Williams, 1987; Giorda et al., 1989; Kimmel & Firtel, 1982). Fifty percent of the total proline residues present in the proteins are found in the repeat regions. This region can form an extended chain in proline–proline conformation, which could be used to project the proteins into the outside of the cell (Early et al., 1988). Thus the similarity due to this particular structure may indicate proteins whose functions may be different but that have to extend outside the cell to function.

The latter idea is supported by lack of homology among the proteins. Computer-assisted amino acid alignment of the deduced 270 proteins shows that the only similarity among the proteins is in the repeat. The other portions of the molecules define products of different single-copy genes. Computer-assisted comparison of the 270-6 deduced protein with the Swiss protein and NBRF protein data bases using the alignment algorithm of Pearson and Lipman (1988) implemented by the UW-GCG sequence analysis software package (Devereux et al., 1984) was also done. It was found that the 270-6 protein is very similar in amino acid sequence to avocado (*Persea americana*) cellulase (Tucker et al., 1987), which is an *endo*-(1–4)-β-D-glucanase (data not shown). The complete avocado protein (495 amino acids) was compared with that region of the 270-6 protein N-terminal to the repeat (460 amino acids) and 36% of the amino acids are identical and 58% represent identical plus conservative changes.

Cellulose is a component of the spore cell wall (Hemmes et al., 1972), and therefore the 270-6 protein may be a cellulase that digests the wall during spore germination, to allow the release of the enclosed amoeba. It would not be required at other stages of development, consistent with the known accumulation of the 270-6 mRNA only during early spore germination (Kelly et al., 1983; Shaw et al., 1986; Figure 1) and its short half-life (Kelly & Ennis, 1985). The other 270 proteins have no similarity to other proteins in the data bank. There is therefore no hint as to their function in slime mold development.

REFERENCES

Cohen, S. M., Knecht, D., Lodish, H., & Loomis, W. F. (1986) *EMBO J. 5*, 3361–3366.

Devereux, J., Haeberli, P., & Smithies, O. (1984) *Nucleic Acids Res. 12*, 387–395.

Dowbenko, D. J., & Ennis, H. L. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1791–1795.

Driscoll, D. M., & Williams, J. G. (1987) *Mol. Cell. Biol. 7*, 4482–4489.

Early, A. E., & Williams, J. G. (1987) *Gene 59*, 99–106.

Early, A., McRobbie, S. J., Duffy, K. T., Jermyn, K. A., Tilly, R., Ceccarelli, A., & Williams, J. G. (1988) *Dev. Genet. (N.Y.) 9*, 383–402.
Giorda, R., & Ennis, H. L. (1987) *Mol. Cell. Biol. 6*, 2097–2103.
Giorda, R., Ohmachi, T., & Ennis, H. L. (1989) *J. Mol. Biol. 205*, 63–69.
Giri, J. G., & Ennis, H. L. (1978) *Dev. Biol. 67*, 189–201.
Hemmes, D. E., Kohma-Buddenhagen, E. S., & Hohl, H. (1972) *J. Ultrastruct. Res. 41*, 406–417.
Henikoff, S. (1984) *Gene 28*, 351–359.
Jaynes, J. B., & O'Farrell, P. H. (1988) *Nature 336*, 744–749.
Kelly, R., & Ennis, H. L. (1985) *Mol. Cell. Biol. 5*, 133–139.
Kelly, L. J., Kelly, R., & Ennis, H. L. (1983) *Mol. Cell. Biol. 3*, 1943–1948.
Kimmel, A. R., & Firtel, R. A. (1982) in *The Development of Dictyostelium discoideum* (Loomis, W. F., Ed) pp 233–324, Academic Press, Inc., New York.
Landschultz, W. H., Johnson, P. F., & McKnight, S. L. (1989) *Science 243*, 1681–1688.
Nellen, W., Silan, C., & Firtel, R. A. (1984) *Mol. Cell. Biol. 4*, 2890–2898.
Pearson, W. R., & Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 2444–2448.
Shaw, D. R., Richter, H., & Ennis, H. L. (1986) *Dev. Biol. 117*, 636–643.
Shi, Y., Son, H. J., Shahan, K., Rodriguez, M., Constantini, F., & Derman, E. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 4584–4588.
Sussman, M., & Brackenbury, R. (1976) *Annu. Rev. Plant Physiol. 2*, 229–265.
Tucker, M. L., Durbin, M. L., Clegg, M. T., & Lewis, L. N. (1987) *Plant Mol. Biol. 9*, 197–203.
Turner, R., & Tjian, R. (1989) *Science 243*, 1689–1694.
Warrick, H. M., & Spudich, J. A. (1988) *Nucleic Acids Res. 16*, 6617–6635.
White, T. J., Arnheim, N., & Erlich, H. A. (1989) *Trends Genet. 5*, 185–189.
Yanisch-Perron, C., Vieira, J., & Messing, J. (1985) *Gene 33*, 103–119.

Evidence for a Structurally Specific Role of Essential Polyunsaturated Fatty Acids Depending on Their Peculiar Double-Bond Distribution in Biomembranes†

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ABSTRACT: ESR spectrometry with 5-, 7-, 10-, and 12-doxylstearate probes and a combined index considering separately the double-bond numbers of essential and nonessential fatty acids were used to investigate the structural role of the double bonds of polyunsaturated fatty esters in membrane phosphoglycerides. Purified brush border membrane vesicles were prepared from the jejunum of piglets receiving either high (HLA) or low (LLA) dietary levels of linoleic acid (18:2 *n*–6). In the LLA as compared to the HLA group, there were no significant modifications of (a) the relative contents of cholesterol, phospholipid, and protein and of (b) the phosphoglyceride class distribution, contrasting with very large changes in the fatty acid compositions of each phosphoglyceride. These changes were characterized by an increase in nonessential monoene and triene (18:1 *n*–9 and 20:3 *n*–9) and a decrease in essential diene (18:2 *n*–6) in LLA- as compared to HLA-fed piglets. The essential tetraene 20:4 *n*–6 remained rather constant despite an overall nonsignificant increase in the LLA group. The total double-bond number (TDBn) was not significantly affected, contrasting with the variations in the double-bond numbers of essential and nonessential fatty acids (DBn_{EFA} and DBn_{nonEFA}, respectively). The combined DBn_{EFA}/DBn_{nonEFA} index was 1.7–3.3 times lower in LLA than in HLA membrane phospholipids. It was concluded that the diet was able to affect the double-bond distribution in the upper and inner half-parts of the membrane leaflet without changing the total number of double bonds. Concomitantly, besides a general decrease in the order parameter with the lipid matrix depth (the order profile), a shape change in the order profile was observed in a comparison of LLA to HLA piglet membranes. Therefore, it was tempting to consider these modified profile shapes as organizational consequences of changes in the double-bond depth-directed distribution. This supports the idea that the position of double bonds in the membrane depth could play a major structural role, providing the essential polyunsaturated fatty acids (EPUFA) with specific features due to their peculiar double-bond distribution and thus emphasizing the "non-eicosanoid" EPUFA function(s).

The most common conformation of lipids in biomembranes is known to be the bilayer structure (Luzzatti, 1968). In the outer faces of the bilayer, cohesion mainly originates both in the covalent anchorage of chains on the glycerol backbone and in the hydrophilic interactions of the head groups. Deeper in the interior of membranes, it is brought about by the hydrophobic interactions between the closest hydrocarbon chains (Lenaz & Castelli, 1985). These interactions near the surface and deeper have mutual stabilizing effects. They are modulated by chemically functional different groups of the phospholipid molecular structure, i.e., the nature of the polar head

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