REVIEW ARTICLE

Protein synthesis during development and differentiation in the cellular slime mould *Dictyostelium discoideum*

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Introduction

Dictyostelium discoideum is a somewhat uncommon species of cellular slime mould (Cavender & Raper, 1965, 1968). It was not described until 1935 when it was found in soils from deciduous woods in North Carolina, U.S.A. (Raper, 1935). Since then the life-cycle of this micro-organism has been much studied and, particularly over the past 10–15 years, has been found increasingly attractive by biochemists seeking to study fundamental aspects of the biochemistry of development and differentiation.

There are two distinct phases—growth and development—in the life-cycle of *D. discoideum* (see Bonner, 1967; Loomis, 1975). Slime mould amoebae continue to grow so long as a bacterial

substrate is available, and the population of single cells increases as the amoebae divide by binary fission. Development is initiated when the bacterial food supply has been exhausted. Amoebae then collect together to form aggregates by a process which involves chemotaxis controlled by the extracellular cyclic AMP concentration (see Newell, 1977) and each aggregate, which may contain up to 10^5 amoebae, secretes a slime sheath (Treffry & Watts, 1976). The aggregates pass through the various morphogenetic stages outlined in Fig. 1 until, finally, each aggregate transforms during 'culmination' into a fruiting body comprising a spore mass surmounting a tall, cellular stalk.

Unlike the original amoebae, the spore and stalk cells are bounded by cell walls. In the spores, the wall comprises three layers. The outer layer contains mucopolysaccharide, the central layer contains cellulose fibres and the inner layer contains

Abbreviation used: DIF, differentiation-inducing factor.

Fig. 1. Morphological stages in development of *D. discoideum*

The time course for development is for the standard conditions described by Sussman (1966). Further descriptions of the life-cycle have been given by Bonner (1967) and Loomis (1975), and the life-cycle has been illustrated by time-lapse photography (Bonner, 1944; Raper & Fennel, 1952; Farnsworth, 1973) and in scanning electron micrographs (Treffry & Watts, 1976; Watts & Treffry, 1976).

proteins and cellulose (Hemmes *et al.*, 1972). The stalk cell wall is a glycogen-cellulose polymer (Ward & Wright, 1965) and the whole stalk is surrounded by a further cellulose sheath (George *et al.*, 1972; Watts & Treffry, 1976). The stalk cells also differ from the spores in being highly vacuolated (George *et al.*, 1972) and necrotic.

Two other groups of cells are also present in fruiting bodies. These are undifferentiated amoebae which collect at the base and apex of the spore mass (Sternfeld & David, 1982) and the cells in the basal disc that attaches the stalk to the substratum. The latter cells resemble stalk cells but are not surrounded by the stalk sheath (George *et al.*, 1972; Watts & Treffry, 1976) and may constitute a third differentiated cell type (Stenhouse & Williams, 1977). A mature fruiting body comprises approx. 70% spores, 18% stalk cells, 10% undifferentiated amoebae and 2% basal disc cells although the proportions of the different cell types can vary between strains (Stenhouse & Williams, 1981). Since there are so few basal disc cells, it seems reasonable to accept the common claim that only two cell types (spore and stalk cells) are formed from amoebae during differentiation.

D. discoideum as a model system of development and differentiation

The main justification for studying development and differentiation in *D. discoideum* is that these processes should be more easily investigated in an organism where only two differentiated cell types are formed than in higher organisms containing many differentiated cell types. *D. discoideum* is therefore seen as a 'model system' of development and differentiation and studies are continued in the expectation that fundamental aspects of development and differentiation will be similar in *D. discoideum* and higher organisms.

The model system also has the advantage that cell growth and development are separated into different phases of the life-cycle. Furthermore, it is fairly easy to grow slime mould amoebae and to induce development that is synchronous.

Growth and development of D. discoideum in the laboratory

Wild-type strains of *D. discoideum* have to be grown with a bacterial substrate such as *Klebsiella aerogenes* (Sussman, 1966), but it is usually more convenient to use mutant strains that can be grown axenically in a simple, semi-defined medium (Watts & Ashworth, 1970) and which are then free of bacterial contamination. So far, defined media for growth of axenic strains (Watts, 1977; Franke & Kessin, 1977) have been little used. The strains in common use are all haploid (Sussman & Sussman, 1962; Robson & Williams, 1977; Zada-

Hames, 1977), which facilitates isolation of mutants since all mutations will be expressed. Genetic analysis is possible and involves exploitation of the parasexual genetics of the micro-organism (Newell, 1978; Welker & Williams, 1982).

Development may be induced by washing amoebae free of nutrients and depositing them on a solid surface, e.g. a Millipore filter overlying a pad saturated with a buffered salts solution (Sussman, 1966). Development is then highly synchronous (Sussman & Lovgren, 1965; Newell *et al.*, 1969a) and fruiting bodies are formed after approx. 24 h. Any particular morphogenetic stage is reached at a predictable time of development (Fig. 1) and it is therefore possible to obtain large numbers of cells at any desired stage of development.

Development and differentiation in D. discoideum

Although the phase of the life-cycle induced by starvation is termed development, two processes—development and differentiation—occur during this phase. Because of this, there seems to be even more confusion concerning the differences between development and differentiation in *D. discoideum* than in higher organisms where development is usually regarded as a gradual process of adaptation to changing conditions in already differentiated cells. Nevertheless, some of the changes that occur in *D. discoideum* following starvation are specific to amoebae destined to become spores, or to amoebae destined to become stalk, and it is these changes that constitute differentiation. Changes that occur in all amoebae on starvation can, by contrast, be considered as development.

Protein synthesis during slime mould development

Introduction

Central to most discussions of development and differentiation is the theory that the two processes are primarily dependent on changes in cell protein synthesis and that these changes are controlled at the level of gene transcription (Gurdon, 1974). This theory has stimulated many of the studies of slime mould development and it is the aim of this Review to assess their success.

Since it has been possible to isolate mutants of *D. discoideum* that are temperature-sensitive for development but not for growth (Loomis, 1969a), it would seem that there are genes specifying proteins that are essential only for development. The problem has been to identify these proteins and to show that their synthesis is exclusive to development and is under transcriptional control. Initially the search for such proteins was amongst enzymes, since these could be easily identified by their cata-

lytic activity, and was concentrated on those enzymes thought to be involved in the new metabolic pathways operating in amoebae during development.

Developmentally regulated enzymes

Glycogen is the only polysaccharide that has been detected in amoebae during growth and early development (White & Sussman, 1963a; Ashworth & Watts, 1970) but, during fruiting body construction, two new polysaccharides appear. Cellulose is synthesized in spore and stalk cells (Ward & Wright, 1965) and an antigenic mucopolysaccharide containing galactose, galactosamine and galacturonic acid (White & Sussman, 1963b) in the spores. The disaccharide trehalose also accumulates in the spores (Ceccarini & Filosa, 1965). The metabolic pathways for synthesis of these carbohydrates (Fig. 2) have not been fully described but studies of mutants lacking UDP-glucose pyrophosphorylase (Dimond *et al.*, 1976), and therefore unable to synthesize UDP-glucose, have confirmed that UDP-glucose is a precursor of all the carbohydrates. Glucose 1-phosphate required for synthesis of UDP-glucose during late development would seem to be derived almost entirely from glycogen accumulated during growth and early development (White & Sussman, 1961).

Some of the enzymes involved in carbohydrate synthesis are virtually undetectable in amoebae during growth and early development, but gradually increase in activity in cells later in development and then decrease in activity when the fruiting bodies have been formed (Fig. 3). The first of these

'developmentally regulated' enzymes to be described was UDP-galactose polysaccharide transferase (Sussman & Osborn, 1964), which catalyses transfer of galactose from UDP-galactose to an acceptor which can be extracted from culminating cells or spores. The enzyme appears to be involved in synthesis of the spore coat mucopolysaccharide. Later studies also identified trehalose-6-phosphate synthetase (Roth & Sussman, 1968), UDP-galactose 4-epimerase (Telser & Sussman, 1971) and glycogen phosphorylase (Firtel & Bonner, 1972) as developmentally regulated enzymes. UDP-glucose pyrophosphorylase, although present in growing amoebae to allow glycogen synthesis, has also been included in this group of enzymes (Ashworth & Sussman, 1967) since it increases markedly in activity in amoebae during development (Fig. 3).

Many other developmentally regulated enzymes have since been described (see Loomis, 1975). A number use amino acids as substrates and may be involved in the amino acid catabolism on which amoebae are dependent for energy production during development. Others are assayed with synthetic substrates and their natural substrates remain largely unknown. Most notable in the latter group are *N*-acetylglucosaminidase (Loomis, 1969b) and α -mannosidase (Loomis, 1970) which are often used as 'markers' of early development (Fig. 3).

In the search for developmentally regulated enzymes, it has been convenient to determine enzyme specific activity (i.e. enzyme activity per mg of total cell protein) at various stages of development. However, a small increase in en-

Fig. 2. Carbohydrate metabolism during late development of *D. discoideum*

Reactions catalysed by developmentally-regulated enzymes are: 1, glycogen phosphorylase; 2, UDP-glucose pyrophosphorylase; 3, UDP-galactose 4-epimerase; 4, UDP-galactose polysaccharide transferase; 5, trehalose-6-phosphate synthetase.

Fig. 3. Some developmentally regulated enzymes in *D. discoideum*

The diagrams are based on data from the following references: (a) —, *N*-acetylglucosaminidase (Quance & Ashworth, 1972); ···, UDP-glucose pyrophosphorylase (Edmundson & Ashworth, 1972); (b) ----, α -mannosidase (Quance & Ashworth, 1972); —, glycogen phosphorylase (Firtel & Bonner, 1972).

zyme specific activity is not necessarily indicative of enzyme accumulation because cell protein content decreases during development by 50–60% (Gregg *et al.*, 1954; Wright & Anderson, 1960; White & Sussman, 1961; Hames & Ashworth, 1974) and an enzyme at constant concentration (i.e. activity per cell) throughout development would increase more than 2-fold in specific activity. This would seem, for example, to account for the increase in β -glucosidase specific activity early in development (Coston & Loomis, 1969), which is not dependent on protein synthesis. To avoid this difficulty, enzymes have usually not

been described as developmentally regulated unless their specific activities increase more than 3-fold during development, but there have been some exceptions [e.g. alanine aminotransferase and leucine aminopeptidase (Firtel & Brackenbury, 1972)].

Differential distribution of developmentally regulated enzymes

Stalk cells are formed from the amoebae at the anterior of the migrating slug (pre-stalk cells), whereas spores are formed from most of the remaining amoebae (pre-spore cells) (Raper, 1940). The amoebae at the anterior of the slug also differ from those at the posterior in ultrastructure (Hohl & Hamamoto, 1969; George *et al.*, 1972), staining properties (Bonner, 1952) and ability to incorporate [3 H]fucose (Gregg & Karp, 1978) and clearly cell differentiation has begun by the slug stage of development. Attempts to determine the extent to which developmentally regulated enzymes are differentially distributed between the pre-spore and pre-stalk cells, and so may be involved in cell differentiation, have been hampered by difficulties in separating the two cell types. Separation has usually been obtained either by dissection or, more recently, by density-gradient centrifugation (Tsang & Bradbury, 1981; Ratner & Borth, 1983).

UDP-galactose transferase, which is required for synthesis of the spore-specific mucopolysaccharide, has been found only in pre-spore cells (Newell *et al.*, 1969a) but, even though trehalose is absent from stalk cells, trehalose-6-phosphate synthetase is present in both pre-stalk and pre-spore cells (Jefferson & Rutherford, 1976a). It would seem that trehalose is synthesized in both pre-spore and pre-stalk cells but is subsequently hydrolysed in the stalk cells as the result of activity of a stalk-cell-specific trehalase (Jefferson & Rutherford, 1976b). The only other cell-type-specific enzyme that has been identified is an isoenzyme of acid phosphatase of unknown function which is restricted to pre-stalk cells (Armant & Rutherford, 1979; Oohata, 1983).

Significance of developmentally regulated enzymes

Increases in the specific activity of all the developmentally regulated enzymes were found to be inhibited in amoebae incubated with cycloheximide to stop protein synthesis and it was therefore generally concluded that accumulation of the enzymes was dependent on protein synthesis *de novo*. Furthermore, the time course of accumulation of many of the enzymes was abnormal in a series of morphologically aberrant, developmental mutants, which tended to confirm that synthesis of the enzymes was under developmental control, and

it appeared that control might be at the level of transcription since accumulation of the enzymes did not occur in amoebae incubated with actinomycin D to inhibit RNA synthesis (see Loomis, 1969c, 1975).

By contrast, Wright and her colleagues obtained results which indicated that the increases in specific activity of many of the developmentally regulated enzymes were not dependent on increased synthesis of the enzyme proteins (see Gustafson & Wright, 1972; Killick & Wright, 1974) and it was suggested that actinomycin D and cycloheximide might not be having their effects on enzyme specific activity by inhibiting protein synthesis (Wright, 1972, but see also Sussman, 1972). Instead it seemed that the enzymes were at constant concentration in cells throughout development and that increases in enzyme specific activity could be detected for the following reasons. (a) The enzymes were unstable in extracts prepared from amoebae early in development but were more stable in extracts of amoebae late in development. (b) The concentrations of tightly-bound enzyme inhibitors decreased in cells during development. (c) During development, inactive enzyme precursors were converted into the active enzymes or enzymes were converted into more active forms.

It would seem at present that only the effects of enzyme inhibitors are of importance and this can be illustrated for alkaline phosphatase. This enzyme, which is a 5'-AMP-specific nucleotidase (Gezelius & Wright, 1965; Armant & Rutherford, 1981), is at low specific activity in amoebae during growth and early development but increases markedly in specific activity during culmination and has all the other properties of a developmentally regulated enzyme (Loomis, 1969b). However, Das & Weeks (1980, 1981) have confirmed earlier indications (Gezelius & Wright, 1965) that the developmental increase in specific activity of this enzyme results from loss of a low molecular weight inhibitor which can bind tightly to the enzyme. When cell extracts were dialysed before assay, high enzyme activity was found at all stages of growth and development. Nevertheless, the developmental increase in alkaline phosphatase specific activity was originally found to be inhibited when amoebae were incubated with cycloheximide or actinomycin D and it is therefore apparent that these inhibitors cannot be used to prove that an increase in the specific activity of an enzyme is the result of protein synthesis *de novo*.

It is possible that further studies will indicate that increases in the specific activity of other developmentally regulated enzymes result from loss during development of enzyme inhibitors, rather than from accumulation of the enzyme proteins, since it would now appear that control of

enzyme activity by specific inhibitors is not uncommon in *D. discoideum*. Inhibitors which seem to control activity of cyclic AMP phosphodiesterase (Gerisch *et al.*, 1972) and two cellulases (Rosness, 1968; Jones & Gupta, 1981) during development are proteins, but the inhibitor of adenylate cyclase (Cripps & Rutherford, 1981) is not inactivated by proteinases. During culmination, 5'-nucleotidase and phosphodiesterase seem to be active only in pre-stalk cells, whereas activity of the cellulases and adenylate cyclase is restricted to pre-spore cells, probably as a result of cell-type-specific loss of inhibitors during development (Brown & Rutherford, 1980; Armant & Rutherford, 1981; Jones & Gupta, 1981; Rutherford *et al.*, 1982a; Rutherford & Brown, 1983).

Nor is it clear that the criteria for recognition of developmental regulation of enzymes are adequate. For example, α -mannosidase and *N*-acetylglucosaminidase have been described as developmentally regulated enzymes, but they can be at as high specific activity in amoebae during growth as during development if the amoebae are in axenic culture (Ashworth & Quance, 1972). Similarly, high concentrations of the developmentally regulated protein discoidin (see the next section) have also been found in amoebae growing axenically (Siu *et al.*, 1976). Burns *et al.* (1981) have argued that growth in the unnatural conditions of axenic culture can induce precocious synthesis of some of the developmentally regulated enzymes because conditions are created in these cultures that, in amoebae grown in more natural conditions with a bacterial substrate, can occur only during development. However, this cannot apply to *N*-acetylglucosaminidase, which is also at high specific activity in amoebae utilizing Gram-positive bacteria as substrates instead of the more commonly used Gram-negative bacteria (Longmore & Watts, 1980). Synthesis of *N*-acetylglucosaminidase appears to be repressed by long-chain fatty acids (K. Longmore & D. J. Watts, unpublished results) which are at high concentration in Gram-negative bacteria but at low concentration in Gram-positive bacteria. It has been proposed that the bacterial cell wall peptidoglycan is the natural substrate for the enzyme and this is present in Gram-positive bacteria at much higher concentrations than in Gram-negative bacteria.

Protein accumulation during development

It would seem that proof that an enzyme accumulates in amoebae during development can be obtained only if the concentration of the enzyme protein is determined. This is possible by use of immunoassay if the enzyme has been purified and an antibody prepared, but this technique has been used to study only a few of the developmentally

regulated enzymes. Developmental increases in the concentrations of the UDP-glucose pyrophosphorylase (Franke & Sussman, 1971; Gustafson *et al.*, 1973), glycogen phosphorylase (Thomas & Wright, 1976) and *N*-acetylglucosaminidase (Every & Ashworth, 1975) proteins were found to be concomitant with increases in the specific activities of the enzymes, but it has been confirmed that specific activity does not correlate well with 5'-nucleotidase (Armant & Rutherford, 1981) or phosphodiesterase (Rutherford & Brown, 1983) concentration.

Immunoassay has also been used to show that the non-enzyme proteins contact site A (Murray *et al.*, 1981; Ochiai *et al.*, 1982) and discoidin (Fig. 4) (Siu *et al.*, 1976) increase in concentration during development. Contact site A is a plasma membrane glycoprotein (Müller & Gerisch, 1978; Müller *et al.*, 1979) required for cell cohesion during aggregation, whereas discoidin comprises two proteins (discoidin I and II) which are lectins (Rosen *et al.*, 1973; Simpson *et al.*, 1974). It would also seem that the spore coat proteins accumulate during late development in the prespore cells and spores (Devine *et al.*, 1982; Wilkinson *et al.*, 1983).

Changes in rates of protein synthesis during development

Even when it had been proved that some proteins accumulate in amoebae during development, it could not be assumed that there were developmental increases in the rates of synthesis of the proteins. Developmental accumulation of a protein could occur, despite an unchanging rate of synthesis throughout growth and development, if

Fig. 4. *Developmental regulation of discoidin I*
The relative levels of accumulation of discoidin I protein and mRNA are shown over the course of development. The curves are adapted from Siu *et al.* (1976), Rowekamp *et al.* (1980) and Rowekamp & Firtel (1980). ---, discoidin I accumulation; —, rate of discoidin I synthesis; ····, discoidin I mRNA accumulation; - · - ·, rate of discoidin I mRNA synthesis (from Poole *et al.*, 1982).

there were a decrease during development in the rate at which the protein was being degraded. To determine whether developmental increases in protein concentration were the result of increased rates of protein synthesis, amoebae were incubated with radioactive amino acids for short periods at various stages of development so that rates of incorporation of radioactivity into proteins of interest could be measured after isolation of the proteins by immunoprecipitation and electrophoresis. In practice, it was not possible to calculate absolute rates of synthesis of the proteins and interpretation of results was not always straight forward.

Franke & Sussman (1973) claimed that accumulation of UDP-glucose pyrophosphorylase late in development results from an increased rate of synthesis of the enzyme protein, but Gustafson *et al.* (1973) concluded from similar experiments that accumulation of the enzyme is due to decreased turnover during development. Other studies have suggested that glycogen phosphorylase (Thomas & Wright, 1976), discoidin (Fig. 4) (Siu *et al.*, 1976), the contact site A glycoprotein (Murray *et al.*, 1981), actin (Tuchman *et al.*, 1974; Alton & Lodish, 1977a) and the spore coat proteins (Fig. 5) (Devine *et al.*, 1982; Wilkinson *et al.*, 1983) are all synthesized at increased rates during the periods when they increase in concentration in amoebae. By contrast, Every & Ashworth (1975) have found that, whereas the developmental accumulation of *N*-acetylglucosaminidase is dependent on protein synthesis *de novo*, the rate of synthesis of the protein during development is no different from that during growth when *N*-acetylglucosaminidase concentration remains low. This would emphasize the importance of not assuming that protein accumulation is indicative of an increased rate of protein synthesis.

Alton & Lodish (1977a) adopted a somewhat different approach. They incubated amoebae with [³⁵S]methionine during growth or at various stages of development and then examined incorporation of radioactivity into about 400 polypeptides that had been separated by two-dimensional gel electrophoresis. Synthesis of about 50 polypeptides was unique to development, synthesis of most of them being initiated during late aggregation (i.e. 10h development). Similarly, protein synthesis has been investigated in the pre-stalk and pre-spore cells of the migrating slug (Alton & Brenner, 1979; Morrissey *et al.*, 1981; Ratner & Borth, 1983) and in spore and stalk cells (Coloma & Lodish, 1981). Synthesis of some polypeptides was cell-type-specific and there were other polypeptides which seemed to be synthesized more rapidly in one cell type than in the other.

Although studies of [³⁵S]methionine incorpora-

Fig. 5. *Synthesis of spore coat proteins during development*

Cells were pulsed-labelled with [35 S]methionine at the different times of development, except for cells harvested at 22–72 h which had been allowed to incorporate [35 S]methionine from 20 h. The spore coat proteins were immunoprecipitated with spore protein antibody and separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Spore coat protein SP66 is the precursor of another protein (SP72) which accumulates during spore maturation (from Wilkinson *et al.*, 1983).

tion have been most helpful in establishing that many changes in protein synthesis occur during development and differentiation, they have given no information about protein accumulation. However, protein accumulation can be assessed from gels that have been stained with Coomassie Blue or silver stains and, for example, Ratner & Borth (1983) were thus able to show that both synthesis and accumulation of a number of polypeptides were cell-type-specific in migrating slugs. Nevertheless, there were a few polypeptides that were at similar concentrations in the pre-spore and pre-stalk cells of the slugs, even though synthesis was cell-type-specific at that stage of development. Presumably, prior to slug formation, these polypeptides were synthesized in all cells.

mRNA synthesis during development

To investigate the extent to which protein synthesis during development is under transcriptional control, Alton & Lodish (1977a) extended their studies to determine whether developmental increases in rates of protein synthesis were concomitant with increases in cytoplasmic mRNA concentration. RNA was extracted from amoebae at various times of development and mRNA concentration was assayed by translation in a protein synthesizing system from wheat germ. Two-dimensional electrophoresis was used to separate the

polypeptides synthesized *in vitro* and many could be identified with polypeptides synthesized *in vivo*. It seemed that, for most of these polypeptides, synthesis *in vivo* was controlled by mRNA concentration. Similarly, developmental changes in the rates of synthesis of actin, discoidin I and discoidin II and the protein inhibitor of phosphodiesterase correlated well with changes in concentration of the respective mRNAs (Ma & Firtel, 1978; Margolskee & Lodish, 1980; Rossier *et al.*, 1983).

Only mRNA that can be translated is estimated by the wheat germ assay system. Any mRNA species found only in developing amoebae by this assay could therefore have been formed either by RNA synthesis *de novo* during development or from untranslatable mRNA precursors synthesized during growth. However, it has been possible to distinguish between these two alternatives for discoidin I mRNA synthesis. Discoidin I mRNA was purified, copied into cDNA and the cDNA incorporated into plasmid DNA. The cDNA could be used to assay total discoidin I mRNA since it was expected to hybridize with both translatable and untranslatable forms of the mRNA. No discoidin I mRNA was found in amoebae growing with a bacterial substrate, but the mRNA was found to accumulate (Fig. 4) during subsequent development in a pattern similar to that found in studies where the wheat germ assay system had been used (Williams *et al.*, 1979, 1980; Rowekamp

et al., 1980; Poole *et al.*, 1982). Thus it is improbable that an untranslatable form of discoidin I mRNA accumulates in amoebae.

Changes in the concentrations of other mRNA species have also been detected by hybridization of the mRNA to cDNA. Only a few mRNA species, including discoidin I mRNA, were found to accumulate during early development (Williams & Lloyd, 1979; Blumberg & Lodish, 1980a). Major developmental changes in amoebal mRNA composition seemed to be restricted to late aggregation (Blumberg & Lodish, 1980a) when there was also a corresponding increase in the complexity of polyadenylated nuclear RNA (Blumberg & Lodish, 1981). By late development, amoebae contain about 4000 mRNA species that are also present during growth (Blumberg & Lodish, 1980b) and a further 3000 species accumulated during development. This would seem to indicate the existence of several thousand developmentally regulated polypeptides in *D. discoideum*, although synthesis of only about 50 has been detected (Alton & Lodish, 1977a). Most of the mRNA species are present in amoebae at low concentration and it is probable that they direct polypeptide synthesis at undetectable rates. However, most of the changes in polypeptide synthesis that were detected occurred at the same stage of development as the major changes in mRNA composition.

Developmental accumulation of new mRNA species would occur if synthesis of the mRNA were initiated during development or if the mRNA were synthesized during both growth and development and were rapidly degraded only during growth. Attempts to demonstrate that nuclei isolated from growing amoebae synthesize mRNA species that accumulate in amoebae only during development have been unsuccessful, although nuclei from developing amoebae were found to synthesize these mRNA species which included discoidin I mRNA (Williams *et al.*, 1979; Landfear *et al.*, 1982; Blumberg *et al.*, 1982). Thus it seems reasonable to conclude that transcription of many genes is specific to development and that there is therefore transcriptional control of synthesis of many developmentally regulated proteins.

mRNA synthesis and differentiation

If differentiation in *D. discoideum* is dependent on differential gene expression, there should be differences between the mRNA species present in the pre-spore and pre-stalk cells of the migrating slug. Some differences have recently been identified (Mehdy *et al.*, 1983; Barklis & Lodish, 1983) but it has yet to be shown that they all arise from cell-type-specific gene expression. It is possible that some of the mRNA species are synthesized in all cells early in development and are then preferentially degraded in either the pre-spore or pre-stalk cells of the slug so as to become cell-type specific. This appears, for example, to account for differences between actin mRNA (and actin synthesis) in pre-spore and pre-stalk cells (Tsang *et al.*, 1982).

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Translational control of protein synthesis

Early studies implied that there was translational as well as transcriptional control of protein synthesis during slime mould development (see Ashworth, 1971) but this was not confirmed by later investigations (Firtel *et al.*, 1973). More recently, translation *in vitro* of mRNA extracted from amoebae during growth and development has identified two examples of translational control. Alton & Lodish (1977b) found that mRNA for five polypeptides was present in amoebae during growth and the first 6 h of development but was translated only during growth, whereas Ramagopol & Ennis (1981) found that mRNA for two ribosomal proteins was present in amoebae throughout the life-cycle but was translated only during development. It is also possible that translational control could lead to the various mRNA species being translated at different rates (Austin & Kay, 1982). However, the overall importance of translational control of protein synthesis in *D. discoideum* has still to be assessed.

Cyclic AMP and control of protein synthesis during development

Identification of cyclic AMP as the chemoattractant controlling aggregation of *D. discoideum* amoebae stimulated further investigations to determine whether this nucleotide might also control other aspects of development, and it is now generally believed that a high cyclic AMP concentration is essential for both spore and stalk cell differentiation (Bonner, 1970; Hamilton & Chia, 1975; Town *et al.*, 1976; Coukell & Chan, 1980; Gross *et al.*, 1981; Kay, 1982). First attempts to determine whether cyclic AMP had any effects on protein synthesis were concentrated particularly on cyclic AMP phosphodiesterase and contact sites A, which were known to appear in amoebae during early development when adenylate cyclase becomes active in catalysing synthesis of the cyclic AMP needed to induce cell aggregation. It was found that both phosphodiesterase and contact sites A were induced precociously in amoebae kept shaken in buffer solutions if cyclic AMP was added at high concentration (e.g. 5×10^{-4} M) or as nanomolar pulses mimicking the natural, pulsatile formation of chemoattractant (Gerisch *et al.*, 1975; Klein, 1975; Town & Gross, 1978; Sampson *et al.*,

1978). The response to cyclic AMP was prevented, at least for phosphodiesterase production, by inhibitors of transcription and translation and this suggested that the effect of extracellular cyclic AMP was ultimately to induce intracellular protein synthesis.

The intracellular concentration of cyclic AMP is increased in amoebae incubated in high concentrations of cyclic AMP owing to diffusion of cyclic AMP into the cells (Sampson *et al.*, 1978), whereas extracellular pulses of cyclic AMP induce an increase in the intracellular cyclic AMP concentration probably by activating the cells' adenylate cyclase (Yeh *et al.*, 1978). Thus it would seem possible for the effects of extracellular cyclic AMP on intracellular protein synthesis to be mediated by increases in the intracellular cyclic AMP concentration and this may apply to induction of contact sites A. However, further studies have shown only a poor correlation between increases in intracellular cyclic AMP concentration and induction of synthesis of phosphodiesterase (Yeh *et al.*, 1978; Lappano & Coukell, 1982) and it has been proposed that it is increases in the intracellular cyclic GMP concentration that control synthesis of this protein (van Haastert *et al.*, 1982; Lappano & Coukell, 1982) and possibly also of contact sites A (Wurster & Bumann, 1981).

The major changes in protein synthesis during *D. discoideum* development occur at late aggregation (10–13h development) when synthesis of many new polypeptides is initiated (Alton & Lodish, 1977a) and this also seems to be affected by the extracellular cyclic AMP concentration. Synthesis of most of the polypeptides stops if aggregates are dissociated (Alton & Lodish, 1977c) but is maintained if cyclic AMP is added at low concentration (20 μ M) to suspensions of the disaggregated cells (Landfear & Lodish, 1980). By contrast, even in the absence of cyclic AMP, disaggregated cells continue to synthesize polypeptides characteristic of pre-aggregation amoebae.

Synthesis of the late-aggregation polypeptides could not be induced by addition of cyclic AMP to cells that had been maintained as single cell suspensions throughout development, although the cells were aggregation-competent. It has therefore been concluded that synthesis of the late aggregation polypeptides can be induced by cyclic AMP only in amoebae that have previously been in tight cell-cell contact (Landfear & Lodish, 1980; Blumberg *et al.*, 1982). However, this may not apply to every one of the late aggregation polypeptides or in all strains of *D. discoideum* (Kay, 1979; Williams *et al.*, 1980; Gross *et al.*, 1981; Kay & Trevan, 1981).

Extracellular cyclic AMP seems to have two effects on synthesis of the late-aggregation polypeptides (Chung *et al.*, 1981; Landfear *et al.*, 1982;

Blumberg *et al.*, 1982; Mangiarotti *et al.*, 1983). First, the half-life of the mRNAs for the polypeptides, which is about 20 min in disaggregated cells, is increased in the presence of cyclic AMP to about 4h which is similar to the half-life of the mRNAs in undisturbed cell aggregates. Secondly, cyclic AMP appears to stimulate transcription of the mRNAs.

Cyclic AMP can also have inhibitory effects on mRNA synthesis; when added to suspensions of amoebae during early development, it blocked accumulation of the mRNAs for discoidin I (Williams *et al.*, 1980) and the protein inhibitor of cyclic AMP phosphodiesterase (Rossier *et al.*, 1983). The effect of extracellular cyclic AMP on accumulation of discoidin I mRNA was shown to result from inhibition of transcription of the mRNA and occurred in conditions which, at the time, suggested that it was mediated by an increase in the intracellular cyclic AMP concentration. However, this conclusion probably needs to be reassessed since more recent studies have indicated that the apparently similar inhibition of synthesis of the phosphodiesterase inhibitor protein is mediated through an increased intracellular concentration of cyclic GMP (Lappano & Coukell, 1982).

By late aggregation, amoebae contain a much reduced concentration of discoidin I mRNA (Fig. 4). A great increase in the concentration of this mRNA can then be induced in amoebae by dissociating the aggregates into single cells (Takemoto *et al.*, 1978; Berger & Clark, 1983), but this effect is abolished if cyclic AMP is added to the disaggregated amoebae. Again, this is consistent with the view that cyclic AMP inhibits discoidin I mRNA synthesis.

All studies seem consistent with a conclusion that, by late aggregation, the extracellular cyclic AMP concentration becomes sufficiently high to induce synthesis of the late-aggregation polypeptides and to inhibit synthesis of some pre-aggregation polypeptides such as the phosphodiesterase inhibitor and discoidin I. The effects of disaggregation can then be considered to result from a decrease in the extracellular cyclic AMP concentration caused by dilution with the buffer used to suspend the single cells. Nevertheless, it has been pointed out that control of mRNA synthesis by cyclic AMP has been demonstrated only in abnormal conditions of development and that there is no unequivocal proof that cyclic AMP has similar effects during normal development (Mangiarotti *et al.*, 1983).

Recent studies, which have shown that it is changes in the intracellular cyclic GMP concentration that best correlate with changes in protein synthesis, might also seem to imply that, if cyclic AMP

does normally control amoebal protein synthesis, it is probably only through changes in the extracellular concentration with cyclic GMP as the intracellular 'second messenger'. On the other hand, developmentally regulated cyclic AMP-binding proteins have been identified in amoebae (Wallace & Frazier, 1979), as well as cytoplasmic cyclic GMP-binding proteins (Mato *et al.*, 1978, 1979), and this would seem to indicate that there are intracellular control mechanisms dependent on the intracellular cyclic AMP concentration. At least one of the cyclic AMP-binding proteins is a protein kinase (Rutherford *et al.*, 1982b).

Cyclic AMP and cell-type-specific protein synthesis

Since an increased cyclic AMP concentration seems to be required for both spore and stalk cell differentiation, it seems improbable that cyclic AMP alone induces the differences in amoebal protein synthesis that ultimately lead to some amoebae differentiating into stalk cells whilst others differentiate into spores. It is possible that stalk-cell-specific protein synthesis is induced by a combination of high cyclic AMP concentration and a low molecular weight, hydrophobic compound that has been called differentiation-inducing factor (DIF) (Town *et al.*, 1976; Kay *et al.*, 1983). DIF, which is synthesized by amoebae only during development (Brookman *et al.*, 1982), seems to be essential for stalk cell differentiation since a mutant deficient in DIF accumulation is unable to differentiate into stalk cells unless given the factor (Kopachik *et al.*, 1983). The mutant is also unable to synthesize the pre-stalk cell specific isoenzyme of acid phosphatase unless it is incubated with DIF (and cyclic AMP), although, in the absence of DIF, it can synthesize other post-aggregative enzymes that are not pre-stalk cell specific. Nevertheless, DIF probably does not have direct effects on protein synthesis in amoebae and there is some evidence that its primary effect is on intracellular pH (Gross *et al.*, 1983).

No factor (other than cyclic AMP) inducing spore differentiation has been described so far. It is therefore possible that all amoebae start to follow the pathway leading to spore differentiation and that some amoebae are then diverted off this pathway by the effects of DIF and differentiate into stalk cells, whereas the remaining cells differentiate into spores. However, sustained cell-cell contact is particularly important for maintenance of the levels of pre-spore specific mRNAs in amoebae (Mehdy *et al.*, 1983; Barklis & Lodish, 1983).

The importance of developmentally regulated proteins

It has clearly been established that synthesis of many proteins is initiated during slime mould

development, but does this necessarily imply that synthesis of these proteins is essential if development is to be successfully completed? This question has been explored particularly by studies of mutants unable to synthesize some of the developmentally regulated proteins.

One mutant that has been isolated could not initiate synthesis of cyclic AMP phosphodiesterase during development and could not aggregate if at low density on the substratum (Barra *et al.*, 1980). Inability to aggregate seemed to be causally related to absence of phosphodiesterase, since aggregation was possible in the presence of added phosphodiesterase although the aggregates were still unable to transform into fruiting bodies. This, together with studies which have shown that amoebae fail to aggregate in the presence of antibody to phosphodiesterase (Goidl *et al.*, 1972), would seem to indicate an essential function for phosphodiesterase during slime mould development.

Amoebae were also unable to aggregate in the presence of monovalent antibody fragments to contact site A (Beug *et al.*, 1970, 1973) and it would seem that synthesis of this protein during development is also essential.

By contrast, studies of other mutants imply that accumulation of several developmentally regulated enzymes is not essential. Mutants with very low UDP-glucose pyrophosphorylase activity throughout growth and development formed fruiting bodies, although this was not possible in mutants totally lacking activity of the enzyme (Dimond *et al.*, 1976). Clearly UDP-glucose pyrophosphorylase is needed during development, but there is apparently no requirement for the developmental increment in enzyme concentration that occurs in wild-type strains (Fig. 3). Fruiting bodies were also formed by mutant strains which totally failed to accumulate *N*-acetylglucosaminidase (Dimond *et al.*, 1973), α -mannosidase (Free & Loomis, 1975) or β -glucosidase (Dimond & Loomis, 1976) during development. However, since β -glucosidase accumulates during culmination (Coston & Loomis, 1969), it could be required for spore germination rather than in formation of fruiting bodies, but spores of the mutant strain have been found to germinate well (Chan & Cotter, 1982).

The ability of mutant strains to form fruiting bodies has been investigated only in laboratory conditions and it is still possible that developmental synthesis of the three glycosidase enzymes and UDP-glucose pyrophosphorylase is essential for development of *D. discoideum* in the less favourable conditions of its natural environment. For example, slug migration is abnormal in *N*-acetylglucosaminidase mutants (Loomis, 1980), possibly because the structure of the slime sheath is abnormal (Watts & Treffry, 1975; Freeze &

Loomis, 1977), and this could be a greater disadvantage in the wild than in the laboratory where slug migration need not occur (Newell *et al.*, 1969b). On the other hand, accumulation of the glycosidase enzymes can also occur in some growth conditions and it is possible that the enzymes are important for growth rather than for development. *N*-Acetylglucosaminidase seems to help growth with Gram-positive bacteria (Longmore & Watts, 1980) and β -glucosidase seems to prevent fusion of cells of the same mating type (Dimond & Loomis, 1976) prior to macrocyst formation which is an alternative to formation of fruiting bodies. Synthesis of α -mannosidase occurs during growth at high culture pH (D. J. Watts, unpublished results) but the enzyme's natural substrate has not been identified.

It has been studies of discoidin I synthesis that have, so far, given most information about control of synthesis of developmentally regulated proteins in *D. discoideum* and it would have been particularly helpful to have found that synthesis of this protein is essential to development. At first it seemed that this hope might be fulfilled, since mutants that failed to synthesize active discoidin I protein were also unable to aggregate, whereas wild-type revertants regained simultaneously the ability to aggregate and to synthesize active discoidin I protein (Ray *et al.*, 1979; Shinnick & Lerner, 1980). However, the characteristics of these mutants are now less easy to understand because further mutants have been isolated that synthesize less than 1% of the normal amounts of discoidin I and II during development and yet are able to aggregate and to form fruiting bodies having viable spores (Alexander *et al.*, 1983). Clearly discoidin I and II are unnecessary for development although there may be some, as yet unidentified, benefits to amoebae in being able to synthesize the discoidin proteins since wild-type revertants arising in stocks of the mutants can then, over a period of months, become predominant.

Since so few mutant strains have been studied, a false impression may have been gained of the extent to which development is not dependent on synthesis of developmentally regulated proteins. A different approach has been to compare estimates of the number of developmentally regulated proteins (several thousand; Blumberg & Lodish, 1980a) with estimates, based on genetic studies, of the number of genes that have an essential function specific to development (150–300; Loomis, 1980). The disparity between the two estimates is so great that, again, there is the implication that, at least for slime mould development in laboratory conditions, many developmentally regulated proteins will be identified for which no essential function will be found.

Conclusions

Investigations of changes in protein synthesis during the life-cycle of *D. discoideum* would seem to confirm that this micro-organism is an appropriate 'model system' for studies of eukaryote development and differentiation. Thus it now seems well-established that new proteins are synthesized at specific stages of development and that synthesis is controlled primarily at the level of transcription of genes into mRNA. Although mutants have been isolated which suggest that some of these developmentally regulated proteins are not essential to development, most of these mutants are deficient in proteins (i.e. the glycosidases, discoidin I and II) for which no role during development has ever been identifiable. Other developmentally regulated proteins have recognized functions during development and it would be expected that, if suitable mutants could be isolated, it would be possible to show that development cannot proceed successfully in the absence of synthesis of these proteins.

The nature of the intracellular signals that control during development the sequence in which genes are expressed, and the duration of transcription of each gene, is still not well understood, but it would seem that significant progress should be made by continuation of studies of the effects on protein synthesis of changes in the concentrations of cyclic AMP and cyclic GMP. Furthermore, the recent purification of DIF (Kay *et al.*, 1983), which appears to be a stalk-cell-inducing morphogen, should facilitate studies of the control of the cell-type-specific gene expression that is the basis of cell differentiation.

Although, at present, we have only a very limited knowledge of the mechanisms controlling gene expression in eukaryotes, there has been great success in identifying these control mechanisms in bacteria, particularly as a result of genetic studies. *D. discoideum* has always seemed an attractive 'model system' of development and differentiation because of the probability that it is an especially suitable eukaryote for analogous genetic studies and, in this respect, the discoidin mutants recently described by Alexander *et al.* (1983) seem particularly interesting. There are four discoidin I genes in *D. discoideum* and at least three are expressed during development (Poole *et al.*, 1981; Devine *et al.*, 1981). In the mutants, there is no discoidin I mRNA synthesis although all the discoidin I genes appear to be intact and this would suggest that it is control of expression of the discoidin I genes that has been deranged. Identification of the lesion in the mutants could therefore be of great help in leading to an understanding of the way in which transcription is controlled in *D. discoideum* so that

expression of some genes is limited to particular times of development. Such studies should be facilitated by construction of cloning vectors for *D. discoideum* and this may be possible from a recently discovered *D. discoideum* plasmid (Metz *et al.*, 1983).

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