Polypeptide compositions of amoebae of the cellular slime mould *Dictyostelium discoideum* separated by partitioning during development

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Amoebae of the cellular slime mould *Dictyostelium discoideum* at 8 h or 10 h development were separated into two populations by countercurrent distribution in a dextran-poly(ethylene glycol), two-phase system. Two-dimensional, polyacrylamide-gel electrophoresis was then used to separate the polypeptides from the populations of amoebae. The two populations of amoebae at 8 h development differed in polypeptide composition as did the populations separated at 10 h development. This confirms that cell differentiation is initiated in *D. discoideum* prior to 8 h development.

When amoebae of the cellular slime mould *Dictyostelium discoideum* are deprived on nutrients, a phase of the life-cycle (development) is initiated during which the amoebae aggregate and go through various morphological stages eventually to form fruiting bodies each comprising a spore mass surmounting a tall, cellular stalk. In standard laboratory conditions (1) the mature spore and stalk cells are formed from amoebae about 24 h after initiation of development, but it is well-established that cell differentiation begins while cells remain amoeboid. Thus the elongate aggregates (migrating slugs) at 16-18 h development have been shown to contain two types of amoebae (pre-spore and pre-stalk cells) (2). Recognition of biochemical differences between the two cell types has been possible largely because the pre-stalk cells are all at the anterior of the migrating slug whereas the pre-spore cells are at the posterior (3) and it has been more difficult to devise methods for detecting any cell differentiation occurring prior to the slug stage of development when aggregates have no obvious polarity. However, two separate populations of cells were obtained when amoebae from aggregates at 9-11 h development were subjected to partitioning in a dextran-poly(ethylene glycol), two-phase system in a thin-layer, countercurrent distribution apparatus (4,5). One population was shown to comprise presumptive spore cells whereas the other contained presumptive stalk cells. Furthermore, even earlier stages of differentiation could be detected by partitioning amoebae immediately after aggregation (8 h development) although the amoebae were not then separated into two discrete populations.

It has been found that many new proteins appear in amoebae during
early development (6,7). It therefore seemed probable that accumu-
lation of some of the proteins would be cell-type specific and that the 
progress of differentiation in amoebae separated by partitioning could 
be assessed by studies of amoebal polypeptide composition. 
Two-dimensional, polyacrylamide-gel electrophoresis has therefore been 
used to study amoebal polypeptide composition and a number of 
differences have been detected between the polypeptide compositions 
of the populations of amoebae obtained after partitioning at 8 h and 
10 h development.

Materials and Methods

Amoebae of *D. discoideum* strain Ax-2 were grown in HL5 medium 
containing 86 mM glucose (8) and were harvested during exponential 
growth. Development was at 22°C on Millipore filters (1). At the 
required times of development, amoebae were washed off the filters 
with distilled water at 4°C.

Amoebae were partitioned between two aqueous polymer solutions 
by using a Bioshef Mark I thin-layer, countercurrent distribution 
apparatus as previously described (4).

Two-dimensional gel electrophoresis, with isoelectric focusing in the 
first dimension and sodium dodecyl sulphate/polyacrylamide-gel 
electrophoresis in the second dimension, was essentially as described by 
(9) but with the modifications described in (10). Amoebae were 
suspended in a solution containing urea (8M), 2-mercaptoethanol (5% 
v/v) and Nonidet P40 (3% v/v) and sonicated. 300 μl was applied to 
the first dimension gel. Amoebae were at 10^7 cells ml^-1 (0 h 
development), 2 x 10^7 cells ml^-1 (8 h development) or 3 x 10^7 cells 
ml^-1 (10 h development) to allow for the marked decrease in protein 
content per cell that occurs during early development (11). 
Ampholines (3% v/v) of pH range 3.5 to 10.0 were included in the 
isolectric focusing gels. The second dimension gels contained 9% 
(w/v) acrylamide and 0.1% (w/v) sodium dodecyl sulphate. Staining 
was with Coomassie blue 0.1% (w/v) in 10% (v/v) methanol/10% 
(v/v) acetic acid. Several gels were run and examined to provide the 
results reported for each time of development.

Results

*Polypeptide composition of amoebae from aggregates at 8 h 
development.*

Amoebae at 8 h development partition to give a broad profile but 
it would seem that amoebae in the leading and trailing edges have 
different fates (4). Amoebae from the trailing edge form spores 
whereas amoebae from the leading edge tend to form stalk.

Examination of the polypeptide compositions of amoebae at 0 h and 
8 h development indicated that there were many changes in amoebal 
polypeptide composition during the first 8 h development, but few 
differential changes could be detected. Thus only three polypeptides 
were found that accumulated in amoebae in the trailing edge of the 
partitioning profile at 8 h development but which were absent from 
amoebae in the leading edge and also from amoebae at 0 h develop-
ment (Figs. 1 & 2a,b). There were also three polypeptides that were 
present in amoebae at 0 h development but which were lost