The effect of tunicamycin on cell-surface changes associated with development and differentiation of *Dictyostelium discoideum*

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Developmental changes in surface properties of *Dictyostelium discoideum* cells have been detected by partitioning. Changes occurring between 0 and 6 h development were not affected by tunicamycin but subsequent changes associated with formation of presumptive spore cells were inhibited. It would seem that only the latter changes are dependent on glycoprotein synthesis.

The cellular slime mould *Dictyostelium discoideum* is much studied as a 'model system' of eukaryotic cellular differentiation since differentiation of this organism gives rise to the formation of only two specialized cell types - spores and stalk cells (1). Much emphasis has been placed on the role of cell-surface changes during the early stages and development and differentiation, particularly with respect to glycoproteins and cell-cell interactions (2,3). Several cell-surface molecules (e.g. Contact Site A (4) and discoidin (5)) that may be involved in cell-cell recognition have been identified, but little is known about the function of these molecules in differentiation.

The approach that we have been using to study the role of cell-surface changes in *D. discoideum* differentiation has been to partition the developing cells between two aqueous polymer solutions. This well-established technique separates cells according to their surface properties (6,7). When the partition steps are carried out in a countercurrent fashion, a distribution of cells is obtained which depends upon differences in cell-surface hydrophobicity and this allows developmental changes in cell plasma-membrane structure to be detected (7).

**Materials and Methods**

A phase system suitable for use with *D. discoideum* cells has been described (8) and consists of 5.5% (w/w) dextran T500, 5.5% (w/w) poly (ethylene glycol) 4000, 0.05 mol/kg NaCl, 0.01 mol/kg Na$_2$SO$_4$, and 0.001 mol/kg potassium phosphate buffer pH 7.8. Thin-layer countercurrent distribution was carried out in a 60-chamber rotor on a Bioshef MkI apparatus as previously described (8).
Cells of strain Ax-2 were grown axenically (9) and harvested in
the exponential phase of growth as described previously (8). Devel-
opment was on Millipore filters at 22° (10). Tunicamycin (a
gift from Dr. J. P. R. Herrmann, Glaxo Research Group) in 0.1%
(v/v) dimethysulphoxide was added to the buffered salts solution (10)
in the Petri dishes containing the Millipore filters to give a final
concentration of 15 μg/ml. At this concentration, tunicamycin from
Glaxo has been found to inhibit glycoprotein synthesis in D.
discoideum without having any inhibitory effects on protein syn-
thesis (11). Cells were treated with tunicamycin in one of two ways: (1)
Cells were put on filters containing tunicamycin at 0 h, harvested
from the filters at different times, and subjected to countercurrent
distribution (CCD). (2) Cells were allowed to develop in the absence
of tunicamycin. Tunicamycin was then added to the filters at
different times of development and the cells allowed to continue
development for a total of 10 h before harvesting and CCD.

Control experiments were conducted simultaneously with amoebae
on filters in the presence of 0.1% (v/v) dimethylsulphoxide. Dimethyl-
sulphoxide at this concentration had no detectable effects on
development.

Results and Discussion

When cells were partitioned 59 times in the phase system on a
Bioshef thin-layer countercurrent distribution apparatus, changes in the
surface properties of cells during the first 10 h of development could
be detected (8). Between 0 and 4 h development, there was an
increase in the surface hydrophobicity of all cells and between 4 and 6
h there was a small decrease in surface hydrophobicity. Between 6
and 10 h development, there was a large decrease in surface hydro-
phobicity in some cells, which distributed separately (peak I) from the
other cells (peak II), which appeared not to have changed in surface
character (Fig. 1) (8). Studies have shown that cells occupying peak
I are presumptive spore cells and the majority of cells occupying peak
II are presumptive stalk cells (8,12). Clearly between 6 and 10 h
development surface changes occur that appear to be related to
differentiation. To determine whether these surface changes involved
glycoproteins, cells were incubated with tunicamycin, which previous
studies had shown to inhibit glycoprotein synthesis in D. discoideum
(13,14), and the partition of tunicamycin-treated and untreated cells
was compared at different times of early development.

When cells were treated by method (1) the resulting CCD profiles
for cells at 2,4, and 6 h development were found to be identical with
those for untreated cells at the same times of development (Fig. 1).
The profile of tunicamycin-treated cells at 8 h development appeared
to be the same as that obtained with untreated cells at 6 h. In
particular this 8-h profile did not show the broadening to the left
(decrease in hydrophobicity) shown by untreated cells at 8 h.
Furthermore, after 10 h treatment with tunicamycin, the profile
consisted of a single broad peak in the same position as peak II of
untreated cells at 10 h and no cells were detected in a position
corresponding to peak I, which is obtained with untreated cells
(Fig. 1).
Fig. 1. Countercurrent distribution of amoebae allowed to develop in the presence of tunicamycin. Amoebae were allowed to initiate development in the presence of tunicamycin (15 μg/ml in 0.1% (v/v) dimethylsulphoxide) and were harvested at the times indicated and subjected to partitioning. In the control experiments amoebae were incubated with 0.1% (v/v) dimethylsulphoxide.

When cells were treated by method (2), with tunicamycin being added at 6 h development, the 10-h CCD profile was similar to that produced by untreated cells at 9 h, i.e. a small peak I and broad peak II. When tunicamycin was added to untreated cells at 8 h, the CCD profile at 10 h was identical with that produced by untreated cells at 10 h.

It has previously been shown that tunicamycin inhibits glycoprotein synthesis in amoebae during early development (11,14). However, it appeared not to prevent the cell-surface changes in hydrophobicity detected by phase partition during the first 6 h of development and this would seem to indicate that these changes are not dependent on acquisition of surface glycoproteins, although changes in plasma-membrane glycoprotein composition occur during this period of development (3). Tunicamycin does inhibit the surface changes...
associated with cells forming a separate population (peak 1; Fig. 1) between 8 and 10 h development and it would appear that these surface changes are dependent on glycoprotein synthesis. Since cells occupying peak 1 are presumptive spore cells (8,12), it is probable that tunicamycin inhibits formation of presumptive spore cells and thus the cell differentiation occurring between 8 and 10 h development. However, it is also possible that presumptive spore cells were formed in the presence of tunicamycin but with altered surface properties so that they could not be distinguished from presumptive stalk cells by CCD. It would seem improbable, however, that prespore cells were present because cells incubated with tunicamycin failed to form fruiting bodies, since development stopped when early aggregates had formed, and clearly tunicamycin does inhibit differentiation.

Tunicamycin inhibits synthesis of some glycoproteins in *D. discoideum* within approximately 15 min of being added (11). However, it was not until about 2 h to 3 h after addition of tunicamycin that effects on the cell surface could be detected. Thus, when added to cells at 8 h development, there was no detectable effect on cell-surface properties at 10 h development but, when added at 6 h development, cells at 10 h development had the surface properties of untreated cells at 9 h development. A possible explanation is that there is a 2-h to 3-h lag between synthesis of some glycoproteins and their insertion in the cell plasma membranes.

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References