SPORULATION-SPECIFIC PROTEIN CHANGES IN YEAST

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1. Introduction

To understand how gene expression is regulated during differentiation requires the identification of biochemical changes specifically associated with the differentiation process. So far there are few eukaryotic systems in which this has been done. Even in a relatively simple system which has received considerable attention, that of meiosis and sporulation in yeast [1–9], relatively few biochemical changes that are specific to sporulating cells have been found [7–9]. Yet from genetic data it has been suggested that approx. 50 loci code for indispensible sporulation functions [10], and for a few genes the timing of their essential expression has been determined [11]. Nonetheless, little is known about the extent to which the genes required specifically for meiosis and sporulation may be read in a sequential way. Many questions about the regulation of gene expression could be answered if a relatively large number of sporulation-associated changes could be directly identified. Attempts have been made to identify polypeptides produced during sporulation by pulse-labelling of sporulating cultures with labelled amino acids, and analysing by polyacrylamide gel electrophoresis the patterns of proteins synthesised [7]. A few changes were detected in this way, but unfortunately the same changes were seen in haploids and diploids homozygous for mating-type, and since neither of these sporulate to any significant extent these changes probably resulted from the shift to starvation conditions and were not specifically associated with sporulation. Using an alternative approach, and the increased resolution of two-dimensional electrophoresis, we have been able to detect changes in proteins which are specific to sporulating cells.

2. Materials and methods

The diploid strain of *Saccharomyces cerevisiae*, JW-1:

$$\frac{a\ ARG\text{-}1\ 7\ his5\text{-}2\ LEU2\text{-}1\ lys1\text{-}1\ URA3\text{-}1\ spd1\text{-}1\ TRP1\text{-}1}{\alpha\ arg4\text{-}1\ 7\ his2\text{-}1\ leu2\text{-}1\ lys1\text{-}1\ ura3\text{-}1\ spd1\text{-}1\ trp1\text{-}1}$$

which sporulates well was compared with another strain, JW-2, which was homozygous (*a*/*a*) for mating type and thus unable to sporulate. (This strain was isogenic with JW-1 apart from the region of the mating-type locus.) Both strains were grown (pre-sporulation phase) for approx. 14–16 h in a defined, low-sulphate minimal medium [12] also containing galactose (2%, w/v) (as a derepressing carbon source in preference to acetate which did not support growth [13]), Na_2SO_4 (10 mg/ml), L-histidine (20 mg/l), L-lysine (40 mg/l), and added folic acid (2 μg/l) and thiamine hydrochloride (400 μg/l) in the presence of $^{35}SO_4^{2-}$ (10 μCi/ml, Radiochemical Centre, Amersham). At a density of 1×10^7 cells/ml, and whilst still growing exponentially, the cells were transferred to sporulation medium (2% w/v, potassium acetate with L-histidine (20 mg/l) and L-lysine (40 mg/l) increasing the density to about 5×10^7 cells/ml. Under these conditions the *a*/α cells sporulated giving approximately 60% mature asci after 24 h.

Samples (1×10^8 cells) of both *a*/α and *a*/*a* cells were removed either as they were transferred to sporulation medium (0 h sporulation) or at intervals following the transfer. They were pelleted and washed with buffer A (5 mM $MgCl_2$, 10 mM Tris–HCl, pH 7.4) at 0°C (3000 × *g*, 5 min). The cell pellet was resuspended in 400 μl fresh buffer A (containing pancreatic RNase (EC 3.1.4.22) and 2 mM

phenylmethylsulphonyl fluoride (PMSF)) at 0°C, and an equal volume of acid-washed 40 mesh glass beads was added. After 2 × 2 min bursts with a vibromix to break the cells, 50 μl of buffer A, containing DNase (EC 3.1.4.6) was added and the mixture allowed to stand for 10 min at 0°C. The sample was then freeze-dried and stored until required for analysis by two-dimensional gel electrophoresis [14]. The freeze-dried sample was taken up in 400 μl of urea sample buffer [14] and after thorough mixing insoluble debris and glass beads were removed by centrifugation (3000 × *g*, 1 min). 15 μl of the supernatant (~300 μl), containing 3–4 × 10^5 cpm as trichloroacetic acid precipitable material, were subjected to isoelectric focusing [14] (pH 3–10) and the resulting gel, after equilibration, was placed on a 5–15% acrylamide slab gel and the proteins electrophoresed in the second dimension in the presence of 0.1% SDS [14]. The slab was 'fixed' overnight in 10% acetic acid before dehydration and impregnation with biphenyloxazole (PPO) scintillant [15,16]. The slab was then dried onto Whatman 3MM filter paper [17] and exposed to Kodak X-Omat XH1 film in a light-tight cassette at −70°C for 7 days. The film was subsequently processed by standard procedures.

3. Results and discussion

From an analysis of pulse-labelled populations of sporulating cultures using density gradient centrifugation, we have found preferential uptake of label by cells which are either not sporulating or which are at an early stage in the sporulation process (manuscript in preparation). We therefore labelled cellular proteins with $^{35}SO_4^{2-}$ only during the presporulation phase of growth, and analysed their subsequent changes in a sporulating and non-sporulating (*a*/*a*) diploid placed under sporulation conditions.

During the period of growth in presporulation medium, label from $^{35}SO_4^{2-}$ is extensively incorporated into both vegetative proteins and amino acids pools. Thus any sulphur-containing proteins synthesised during the subsequent sporulation of these cells would also be labelled with either the free amino acids or those released by the breakdown of labelled proteins. Furthermore, should sporulation involve the specific modification of pre-existing vegetative polypeptides the new forms would also be labelled. Hence by this approach changes during sporulation due to either modification or de novo synthesis would be detectable.

The prelabelling resulted in steady-state labelled cells with an uptake of less than 50% of the isotope (data not shown). The subsequent extraction of polypeptides, allowing for losses due to the counting methods and trichloroacetic acid precipitation gave ~50% recovery of this isotope in 300 μl of supernatant. The remainder could be accounted for in unrecovered urea sample buffer, insoluble cell debris and adhesion to glass beads.

By comparing the autofluorograms from duplicate experiments we have found a total of 45 significant changes, i.e. 11% of the 400 most prominent polypeptides detected (fig.1D), during 24 h in sporulation medium. Of these, 23 resulted from a marked alteration in the relative amount of particular polypeptides (observed as intensity changes in spots) indicating possible control at the point of production, while the remainder were new appearances or complete disappearances. In the 24 h period there were 7 appearances and 11 increased in spot intensity which were common to both *a*/*a* and *a*/α cells and therefore probably the result of general adaption to the sporulation medium. However a further 13 new spots and 7 increases in spot intensity were apparent only in the sporulating *a*/α cells, indicating that these 20 were specifically associated with the sporulation process. In addition other interesting changes occurred (fig.1D). One spot (A_7) disappeared completely from *a*/*a* cells only, and two more (A_1 and A_5) were reduced in intensity. Similarly one spot (A_4) disappeared completely and two (A_2 and A_3) were reduced in intensity in both *a*/α and *a*/*a* cells. Furthermore, one new spot (A_6), although appearing in both *a*/α and *a*/*a* cells, was much more evident in the *a*/α cells. The nature of these changes and their implications is currently being studied.

By examining extracts of cells from 4 and 10 h in sporulation medium (data not shown) we have noticed differences in the timing of various changes. However more detailed analysis, including the use of sporulation mutants, is needed to determine the complete sequence of these changes.

The above alterations could be due to de novo synthesis of proteins and/or to specific modifications of

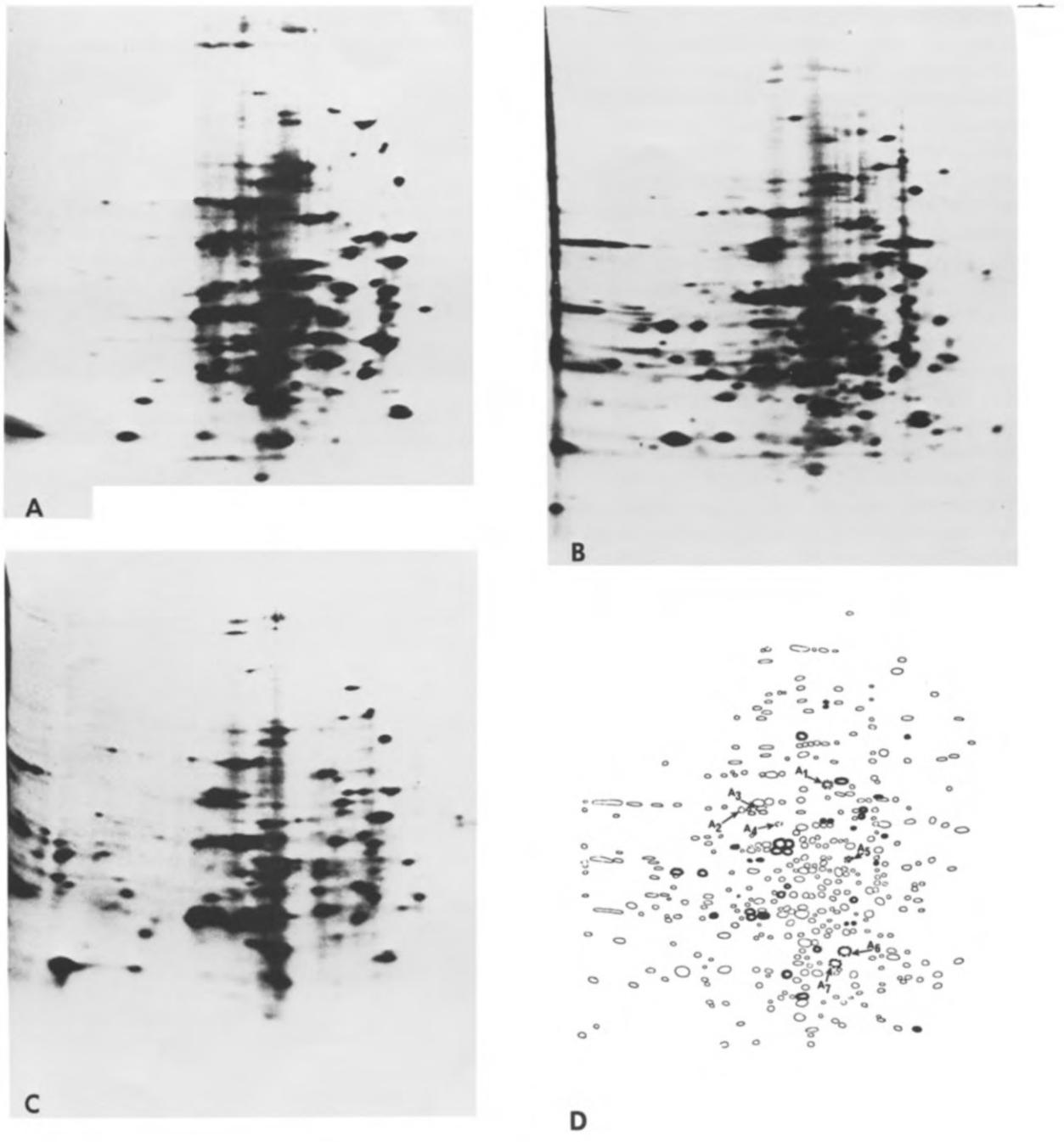

Fig. 1. Examples of autofluorograms of ^{35}S-labelled proteins separated in the first dimension by isoelectric focusing (pH 3–10) and in the second according to molecular weight in a 0.1% SDS, 5–15% polyacrylamide gel slab [14]. Proteins from a/α 0 h sporulation cells (A), a/α 24 h sporulation cells (B) and a/a 24 h sporulation cells (C) are shown, with a composite diagram (D) of the most prominent polypeptides showing: common (heavy outline) or sporulating cell-specific (filled spots) appearances, and other changes (A_1–A_7) over the 24 h sporulation period.

existing proteins in terms of size or charge, and we are currently distinguishing which changes fall into each category.

The results are unlikely to be artefacts of proteolysis since the use of buffers above pH 7.0 and the presence of a protease inhibitor (PMSF) should reduce this to a minimum during extraction [18]. Furthermore, controls were examined in which unlabelled cells from 24 h sporulation cultures (both a/α and a/a) were co-extracted with the respective $^{35}SO_4^{2-}$-labelled 0 h sporulation cells. (Data not shown.) No significant differences were detected when compared with previous 0 h extracts. This showed that the changes observed were not artefacts due to proteolysis during the extraction procedure.

In summary, 18 changes in cellular polypeptides occurring during meiosis and sporulation, and specifically associated with one or the other of these processes, have been detected by a method capable of resolving clearly about 400 polypeptide species. This is a significant number of changes, be they the result of de novo synthesis or modifications to existing proteins and the technique presented here will prove of considerable value in analysing the biochemical changes occurring during meiosis and sporulation, and the mechanisms controlling these processes.

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