Short Communication

Posttranslational Regulation of Repressible Acid Phosphatase in Yeast

Martin Ernst Schweingruber and Anne-Marie Schweingruber
Institute of General Microbiology, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland

Summary. On the basis of genetic data it has been suggested that repressible acid phosphatase of *Saccharomyces cerevisiae* is regulated by a control circuit involving operator-repressor mechanisms (Toh-e et al., 1978).

We measured no significant difference in the amount of translatable mRNA of repressed and derepressed cells in the reticulocyte in vitro translation system. We found a 25 fold difference in specific enzyme activity in repressed versus derepressed cells whereas the amount of $^{35}$S-methionine labelled enzyme protein as measured by antibody precipitation varies only 2-3 fold. This argues for posttranslational regulation of preexisting inactive acid phosphatase. Minor regulatory effects at the transcriptional or translational level cannot be excluded.

Inducible and repressible genes in procaryotes are known to be regulated at the level of transcription. In eucaryotes the phenomenon of enzyme induction and repression is also known, but the biochemical mechanisms involved are still poorly understood. An example of a repressible enzyme in a eucaryote is the acid phosphatase of yeast. Two isoenzymes of acid phosphatase have been described in the wildtype H-42 of *Saccharomyces cerevisiae*. One is constitutive and the other is repressible by inorganic phosphate. The expression of the structural gene *pho E* of the repressible acid phosphatase is regulated by four other genes, *pho B*, *pho R*, *pho S*, and *pho U*. These regulatory genes may act via a control locus, contiguous with the *pho E* locus. A control circuit involving operator-repressor control mechanisms has been proposed to explain the genetic data (Toh-e et al., 1973; Toh-e and Oshima, 1974; Ueda et al., 1975; Toh-e et al., 1975; Toh-e et al., 1978). In order to elucidate the biochemical mechanism of the proposed transcriptional control of repressible acid phosphatase in *S. cerevisiae*, we programmed a reticulocyte in vitro translation system with total RNA or poly A-RNA extracted from repressed as well as derepressed cells. We measured the amount of translatable mRNA for repressible acid phosphatase by precipitating the in vitro translated acid phosphatase by pure antibodies specific for repressible acid phosphatase. We did not find any significant difference in the amount of mRNA from repressed or derepressed cells even though the specific activities of the enzyme differed more than 20 fold (data not shown). From this we concluded that derepression may not occur by a mechanism that drastically alters the relative amount of translatable mRNA for acid phosphatase. Here we report experiments which indicate that the relative increase in activity of repressible acid phosphatase upon phosphate starvation is mainly due to activation of preexisting inactive phosphatase.

In order to make antibodies, repressible acid phosphatase was purified to homogeneity and injected into rabbits. Antibodies were purified and shown to be specific for repressible acid phosphatase (Fig. 1). We found no cross-reactivity with bovine serum albumin, *S. cerevisiae* invertase (an extracellular glycoprotein very similar to repressible acid phosphatase) nor with repressible acid phosphatase from the fission yeast *Schizosaccharomyces pombe*. The same specificity was also obtained with serum (data not shown). The antibodies were used as a probe to detect $^{35}$S-labelled acid phosphatase in cell extracts of cells grown under repressing and derepressing conditions. In crude extracts from repressed versus derepressed cells, there is a 25 fold difference in specific enzyme activity whereas the amount of enzyme protein, as measured by antibody precipitation, varied only 2-3 fold. The precipitation curve with increasing amounts of antiserum is similar for extracts from repressed and derepressed cells. In both extracts the immunoprecipitable protein can be competed out with active acid phosphatase (Fig. 2). Similar immunoprecipitation results were obtained with $^{3}$H-leucine labelled cells. The percentage of precipitated radioactivity from $^{3}$H-leucine...
Fig. 1. Competition experiments illustrate the specificity of antibodies against homogeneous repressible acid phosphatase of *S. cerevisiae*. The antibody precipitation reactions were performed in 1.5 ml Eppendorf plastic tubes. These contained in 100 μl of phosphate-buffered saline (PBS) 3 μg of 14C-labelled repressible acid phosphatase protein, 30 μg pure antibodies and variable amounts of competitor protein as repressible acid phosphatase of *S. cerevisiae* (●), invertase of *S. cerevisiae* (○), repressible acid phosphatase of *Schizosaccharomyces pombe* (□) and bovine serum albumin (○). Incubation occurred for 1 h at 37°C and subsequently for 15 h at 4°C. The immunoprecipitate was washed as described (Maurer et al., 1976), the final pellet was dissolved in 0.5 ml 1M acetic acid and counted in 8 ml aquasol. Repressible acid phosphatase from the wildtype H-42 of *S. cerevisiae* was purified to homogeneity as described (Dibenedetto and Cozzani, 1975). The criteria for judging homogeneity are those cited in ref. Dibenedetto and Cozzani. In addition it was shown that upon removal of the carbohydrate moiety by HF (Mort and Lamport, 1977) the deglycosylated protein ran as a sharp single band on SDS polyacrylamide gel (M.E. Schweingruber, in preparation). The purified repressible acid phosphatase was labelled *in vitro* by reductive alkylation (Rice and Mans, 1971) yielding a specific activity of 1.5·10⁵ c.p.m./mg acid phosphatase protein. For the immunoprecipitation experiments it was diluted to a specific activity of 400 c.p.m./μg protein. Repressible acid phosphatase of *S. pombe* and invertase of *S. cerevisiae* were purified as described (Dibenedetto and Cozzani, 1977; Trimble and Malley, 1977). Antibodies against repressible acid phosphatase of *S. cerevisiae* were raised by 3 consecutive subcutaneous injections into a rabbit. At intervals of 2 weeks 500 μg repressible acid phosphatase dissolved in 1 ml saline and 1 ml Freund's adjuvant complete was injected. After six weeks the rabbit was bled. The crude gammaglobulin fraction was prepared from the serum as described (Palmiter et al., 1971) and the antibodies specific for repressible acid phosphatase were isolated by affinity chromatography on repressible acid phosphatase immobilized on a CNBr-activated Sepharose 4B column. 30 mg of enzyme was coupled to 3.5 ml swelled activated Sepharose as indicated by the manufacturer (Pharmacia). 8 ml of the crude gammaglobulin fraction containing 13 mg protein/ml were applied to the column. The column was washed with 20 ml PBS. 33 mg IgG were retained and eluted with 30 ml 3M NaSCN and subsequently with 12 ml HCl-Glycine 0.1 M pH 2.5 containing 10% dioxane. A total of 25 mg pure IgG were recovered and extensively dialyzed in PBS. Protein concentrations were measured by the method of Lowry et al. (1951) with crystalline bovine serum albumin as a standard.

Fig. 2. Immunoprecipitation of repressible acid phosphatase from extracts of cells grown under repressing (○) and derepressing (□) conditions. To repress the acid phosphatase cells were grown in YEPD and to derepress, they were grown in YEPD with inorganic phosphorus removed (YEPD-P) (Rubin, 1973). 100 ml cultures were shaken in 500 ml Erlenmeyer flasks at 30°C with 0.15 mCi 35S-methionine (800 Ci/mMol). At a density of 1·10⁷/ml the cells were washed with 100 ml 0.1M acetate pH 4.5. They were resuspended in 10 ml 0.1 M acetate pH 4.5 containing 1% Triton and disrupted with 20 g glassbeads (0.45 mm diameter) for 45 s in a Brown homogenizer. Cell debris were spun for 30 min at 27,000 g. The specific activity of the extract prepared from the derepressed cells was 0.45 μg/mg protein, and 25 fold lower for the extract from repressed cells. (The definition of the enzyme units (U) and the assay conditions for acid phosphatase are described in ref. Schurr and Yogil). 5 ml supernatant was dialyzed in 51 PBS for 16 h. The specific radioactivities from the extracts prepared from repressed and derepressed cells were 1·10⁵ c.p.m./mg protein and 1.6·10⁵ c.p.m./mg protein respectively. A small precipitate was formed during dialysis. It was pelleted at 10,000 g for 10 min and redissolved in 1 M acetic acid. It constituted 1.5% of the total counts of the repressed cells and 1.8% of the total counts of the derepressed cells. Each immunoprecipitation was done with 100 μl supernatant and variable amounts of specific antisera as indicated on the abscissa. The total reaction volume was brought to 400 μl with PBS. Incubation conditions, washing and counting was as described in legend of Fig. 1. Competition experiments were performed in reaction mixtures containing 50 μl serum, 100 μl extract from derepressed (●) or repressed (○) cells, 250 μl PBS and variable amounts of purified repressible acid phosphatase. The results are given in the insert of Fig. 2.