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Effects of biotin on growth and protein biotinylation in *Saccharomyces cerevisiae*☆

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Abstract

In mammals, biotin, well known for its role as the cofactor of carboxylases, also controls the expression not only of proteins involved in this function, but also of a large number and variety of other different proteins. As a first step towards looking for a rationale for these phenomena, we intend to compare these regulatory functions of biotin between the rat and the much less evolutionized eukaryote, *Saccharomyces cerevisiae.* Thus far, we have measured growth in yeast cultured on different concentrations of biotin to choose the experimental conditions to be used (2, 200 and 2000 μM) and have found that a band corresponding to the biotinylated *S. cerevisiae* Arc1p protein appears at streptavidin Western blots at a biotin concentration above 2000 μM, its density increasing with higher biotin amounts. We will now study changes in yeast transcriptome with these varying concentrations and compare them with changes observed in the rat.

Keywords: Biotin; *Saccharomyces cerevisiae*; Yeast; Biotinylated; Carboxylase

1. Introduction

For a long time it was considered that the only function of mammalian biotin was as a prosthetic group of carboxylases [1], whereas in prokaryotes an additional role as coregulator of the *bio* operon was recognized [2,3]. Recently, we discovered that biotin regulates the amount of holocarboxylase synthetase mRNA in various rat's organs, as well as the protein amounts (masses) of the mitochondrial carboxylases [4], findings that were corroborated by Solorzano-Vargas et al. [5] in human cell lines.

More enigmatic are the effects of biotin on the expression of hundreds of mammalian genes and proteins seemingly unrelated among themselves, and to the processes involved in carboxylation [6,7]. Some of them are very important in carbohydrate metabolism, especially inducing glucokinase (GLK) [8–11] and repressing phosphoenolpyruvate carboxykinase (PEPCK) [12], effects mediated by soluble guanylate cyclase (sGC) and cGMP [13] at transcription.

It has been reported that biotin binds covalently to histones [14–17] and that the degree of histone biotinylation correlates with functional cellular activities, such as cell proliferation, gene silencing and the cellular response to DNA damage [16,18]. This may be an epigenetic mechanism by which biotin exerts its regulatory functions, intervening in the remodelation of chromatin.

It seems unlikely that this enormously wide range of proteins being controlled by biotin is restricted to the mammalian *phylum*. Given the dynamics of histone biotinylation [18], it is plausible that the diversification of regulatory targets for biotin might have been associated with the emergence and evolution of eukaryotes, i.e., with the origin of chromatin and histones. Thus, we are interested in

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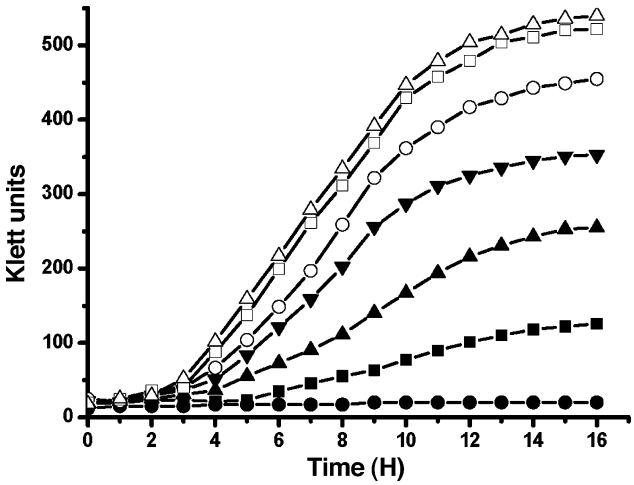

Fig. 1. Growth curves of *S. cerevisiae* strain BY4741 in minimal medium with glucose as the carbon source and different biotin concentrations. Biotin concentrations were as follows: ●, 0; ■, 0.2 μM; ▲, 2 μM; ▼, 20 μM; ○, 200 μM; □, 2000 μM; and △, 20000 μM. For details see Materials and methods.

looking for these phenomena in the simple eukaryote *S. cerevisiae*. There are many examples of functions present in yeast that have been conserved up to humans, and their comparisons have proven valuable to identify and understand basic biological mechanisms. The general structure of chromatin has been found to be very similar in all eukaryotes. Assuming that histone biotinylation is a principal mechanism mediating the regulatory actions of biotin [18], it may have been incorporated early in evolution into the control of chromatin.

We initialized a project to study whether there are similar regulatory effects of biotin in *S. cerevisiae*. Thus far, we determined whether biotin concentrations in culture media affect growth rates and protein biotinylation. With this information, we will later choose the best biotin amounts to use for the identification of those transcripts that might be substantially changed.

2. Materials and methods

2.1. Reagents and chemicals

Nitroblue tetrazolium chloride, BCIP, streptavidin-alkaline phosphatase conjugate and biotinylated sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis high-range standards were purchased from Bio-Rad (Mexico City, Mexico). Complete protease inhibitors were purchased from Roche Molecular Biochemicals (Mexico City, Mexico).

2.2. Yeast strains and media

The *S. cerevisiae* strain used in this study was BY4741 (Mat a: his3Δ1; leu2Δ0; met15Δ0; ura3Δ0). Yeast cells were grown routinely in YPD medium (1% Bacto-yeast extract, 2% Bacto-peptone and 2% glucose). For the growth experiments, biotin-free minimal medium was prepared according to the Difco manual, and different amounts of biotin were added to it for each vitamin concentration. Both the yeast strain and the vitamins were a gift from Dr. Alicia Gonzáles (Instituto de Fisiología Celular, UNAM, Mexico). Amino acids were purchased from Sigma.

2.3. Growth experiments

Precultures were prepared by inoculating a small amount of yeast cells into 100 ml minimal medium in an Erlenmeyer flask, which was agitated at 250 rpm at 30°C for 24 h [19]. Cells were inoculated at an $OD_{540\ nm}$ of 0.03, into 100 ml minimal medium with glucose (fermentation conditions) or lactate (respiration conditions) as carbon sources, and containing biotin at 0, 0.2, 2, 20, 200, 2000 and 20000 μM. Results were obtained at every hour using a Klett-Summerson spectrophotometer.

2.4. Biotinylated protein determination

S. cerevisiae cells were grown to mid-logarithmic phase in minimal medium with different biotin concentrations. Harvested cells were broken using a Braun cell homogenizer and 0.45-mm-diameter glass beads, as reported previously [20] in a cell lysis buffer containing 100 mM Tris–HCl, 10 mM KCl, 8% glycerol, 1 mM DTT, 1 mM PMSF, pH 7.8 [21]. Homogenates were centrifuged (27000×*g*, 10 min), the supernatant was again centrifuged (154000×*g*, 30 min) and the new supernant was assayed by SDS-PAGE and Western blotting with streptavidin–horseradish–peroxidase conjugate (Amersham Biosciences) as described before [22].

3. Results and perspectives

Fig. 1 shows the growth response to different biotin concentrations when the carbon source was glucose; yeast grew slower when lactate was the carbon source (results not shown). There was some growth at biotin concentrations as low as 0.02 μM. No further growth was observed above 2000 μM. Therefore, we plan to use 2 μM as a biotin-

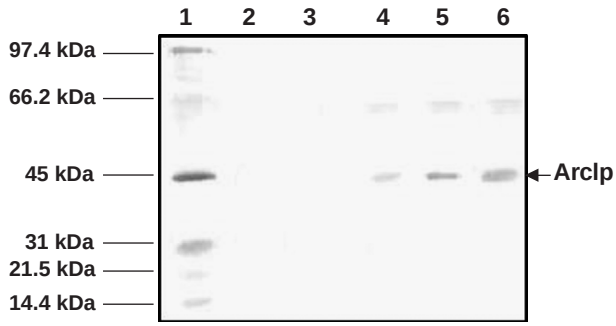

Fig. 2. Degree of biotinylation of *S. cerevisiae* Arc1p protein (MW 45 kDa) as a measure of the biotin status of the cells. Lane 1, molecular weight standard; lane 2, 2 μM biotin; lane 3, 20 μM biotin; lane 4, 200 μM biotin; lane 5, 2000 μM biotin; and lane 6, 20000 μM biotin.

deficient medium, 200 μM as the "physiologic" concentration and 20 000 μM as an excessive concentration. We determined the degree of biotinylation of *S. cerevisiae* Arc1p protein [23] by means of streptavidin blots, as a measure of the biotin status of the cells. As can be seen in Fig. 2, the band corresponding to this protein (MW 45 kDa) appears at a biotin concentration above 200 μM, and its density increases with higher biotin amounts. We will next study changes in yeast transcriptome with these varying concentrations and compare them with changes observed in the rat.

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