Yeast cyclic AMP concentration can vary 70-fold

The sole role of adenosine 3':5'-cyclic monophosphate (cAMP) in cells of the yeast *Saccharomyces cerevisiae* has been proposed to be that of a biochemical switch whose concentration directly controls entry into, and probably exit from, stationary phase via regulation of the activity of protein kinase A (7). Such a role for cAMP would be best achieved by having similar, low levels under starvation conditions and similar, high levels under growth conditions, with intermediate values excluded since these would give conflicting signals to the cell. By mutating genes involved in the regulation and biosynthesis of cAMP it has been possible to create cells with either very high or very low concentrations of cAMP. Studies of such cells have produced results which are consistent with these suggestions (7).

Two recent papers in *Nature* (1, 6) suggest that cAMP concentration inside yeast cells has an additional function, namely controlling the overall expression of cyclin (*CLN*) genes. Since cyclin genes are involved in initiation of the cell cycle at *start*, and have a role in determining cell size at *start*, this new proposal means that cAMP indirectly couples cell cycle progression to growth (biomass). Such a role would be consistent with cAMP levels showing a wide range of concentrations dependent on external conditions such as growth rate, nature of the carbon source and/or substrate concentration. One of the papers referred to the lack of suitable measurements of intracellular cAMP concentrations in wild-type cells grown under a wide range of conditions (6).

We have grown yeast cells in a variety of media and measured intracellular cAMP concentration during exponential growth (as judged by constant rate of growth of the population and a constant fraction of budded cells). We found that there was an inverse linear relationship between cell density and intracellular cAMP content of cells which varied at least 70-fold (Fig. 1). Since cells were inoculated into these media to give approximately the same initial cell density, higher cell density at *start* is relatively constant at slower growth rates and only increases noticeably at the fastest growth rates, it is likely that the role of cAMP in determining cell size is more complex than has been suggested in either of these papers. To resolve the issue it would be desirable to control independently the level of both cyclin proteins and cAMP, and determine the effect on cell size.

**Fig. 1.** Intracellular cAMP concentration and cell density in batch cultures. Flasks of complex (YP) or synthetic minimal (S) media (4) containing 2% (w/v) D-glucose (D), fructose (F), glycerol (G) or pyruvate (P), were inoculated with an exponentially growing culture of a diploid prototroph (D1) (3) that had been growing at 25°C in SD medium. The cells were then incubated at 25°C in a shaking water bath. Samples were taken for cell number and cAMP concentration measurements (2) when the cells had reached mid-exponential phase (constant fraction of budded cells).

Extracellular glucose concentration is known to affect intracellular cAMP concentration (3). Our data suggest that this kind of relationship extends to other carbon sources. Although the correlation between cell density and cAMP differs between fermentable and non-fermentable carbon sources, the difference is less than twofold under other otherwise identical conditions. The range of media that were used support a wide range of growth rates but this was not reflected in a consistent relationship to cAMP concentration.

The results are compatible with the earlier biochemical switch model only if cells are particularly sensitive to certain threshold levels of cAMP. The data are fully congruent with the new proposals on the additional role of cAMP but suggest that cAMP concentration continually reflects available external nutrient supply rather than simply growth rate or carbon source as was proposed (1, 6). Since it is known that cell size at *start* is relatively constant at slower growth rates and only increases noticeably at the fastest growth rates, it is likely that the role of cAMP in determining cell size is more complex than has been suggested in either of these papers. To resolve the issue it would be desirable to control independently the level of both cyclin proteins and cAMP, and determine the effect on cell size.
The discovery of 'cytochrome o'

In 1954, Lucille Smith, LaRoy Castor and one of us (B.C.) discovered a photos dissociable carbon monoxide compound in several types of bacteria, the absorption bands of which were similar to haemoglobin. In view of this similarity, and recognizing the claim by Keilin & Tissiére of the presence of haemoglobin in certain micro-organisms, functional studies were made. Using an improvement of Warburg's photochemical method, Castor & Chance (2) showed the mysterious haemoprotein to be an oxidase and named it cytochrome o. It is important to recognize that the name, o, signifies oxidase. In an otherwise very clear summary of haem nomenclature (1), an explanation of 'cytochrome o' illustrates the present misunderstanding of the name's origin: "The original designation of an a-type cytochrome meant that it contained a heme B, which can bind to carbon monoxide (or O2, extrapolating for oxidases)." Firstly, the name was intended to describe function (o for oxidase), not haem type. At the time, haem O (see below) had not been discovered but the spectral similarity with haem B (5) justified the supposition that the ligand-reactive haem was B. Secondly, this description implies that the characteristic of cytochrome o is CO binding, whereas Chance & co-workers coined the name only after the oxidase function, i.e. the reaction with oxygen, was demonstrated by the light reversibility of inhibition of respiration (2). The direct demonstration of the binding of oxygen with cytochrome o to give a compound spectrally resembling the CO compound was obtained by us only much later (6), with the availability of low-temperature photolysis methodologies.

A new era of oxidase research......

The explosion of interest in research on bacterial oxidases is largely the consequence of molecular cloning of structural genes, purification of oxidase complexes and the recognition of a much greater diversity of oxidase types than had hitherto been suspected. In addition to the long recognized existence of oxidases containing cytochromes a, a1, a2, d and d1 as ligand-binding haems, it has become clear that cytochrome b can act in this capacity and that certain oxidases contain previously unrecognized haem types. For example, the cytochrome s-type oxidase of Escherichia coli is now known to contain a novel haem, haem O (8). To add to the possibility of confusion (or interest, depending on one's point of view) it is now known that a haem site in an oxidase protein can be occupied by more than one class of haem. For example, in the cytochrome s-type oxidase of E. coli, a variant of the oxidase is known in which both haems are the O-type, in contrast to the normal oxidase in which one haem is O (ligand-binding) and the other is B (9).

Quite naturally, those working on these oxidases have sought a way of distinguishing, in the name, between haems in a given oxidase. It is particularly valuable to demonstrate in the name which of the haems is, or are, capable of binding oxygen (the substrate) and other ligands such as CO and cyanide. Precisely this requirement arose in the 1930s, when Keilin (3) described 'two...compounds having the same haem nucleus. One of these compounds (a) is not autoxidizable and does not combine with potassium cyanide or carbon monoxide, while the other (a') is autoxidizable and combines with both'. Why was the name a chosen? Again, Keilin himself makes this perfectly clear. We shall designate this new component as a in order to distinguish it from cytochromes a1 (589 nm) and a2 (630 nm) which are known to occur only in a few bacteria devoid of cytochrome o.' (We know now that certain bacteria do possess cytochrome a2 as well as a1, but the principle of nomenclature remains valid.) Cytochrome a2 was the name given to a cytochrome observed in Aerobacter and a few other bacteria. This name is now little used because in many bacteria, but not all (10), cytochrome a2 has been shown to be a high-spin s-type cytochrome that is a component of the cytochrome bd complex. Cytochrome a2 was the early name for cytochrome d. Like most types of a1, it was subsequently shown not to contain haem A but, in this case, a chlorin haem that gives rise to the unique spectral features of this type of oxidase.

....but do we need new names, and are some of them really new?

It is with supposed deference to the name 'as', that the names bo, bb, and others have been adopted by some workers in this field over the past few years (for a survey, see 10). The subscript s denotes the ligand-binding haem. The name was first used for the E. coli cytochrome s-type oxidase by Puustinen & Wikström (8), who suggested that this oxidase be called bs (when the haems are B and O) or os (when both haems are O). It is claimed that the nomenclature is useful because it shows the haem types (we agree), that it shows which is the ligand-binding haem (it does, but a system for doing so already exists, see below), and that it is 'historically pertinent' (we disagree). If 'a' has the same etymological root as ao, where are cytochromes a1 and a2?

We accept that the subscript is useful in signifying which haem binds oxygen. This is a particularly valuable feature in cases like cytochromes ao and bb where the low-spin (electron-conducting) and high-spin (ligand-binding) haems are of the same chemical structure differing only in their axial ligation to the protein and thus their ability to bind oxygen. However, there exists already an internationally validated recommendation (4) that serves precisely this function. It is simple and unambiguous, namely that the 5-coordinate ligand-binding haem is primed as 3h (for the E. coli oxidase) and 3d (for the mitochondrial oxidase).

Unfortunately, this nomenclature has never 'caught on' amongst students of the mitochondrial oxidase, probably because the official recommendation lagged many years behind the