PN 1288

Stimulation of lipid synthesis by yeast ribosomal preparations

We have reported that optimal synthesis of fatty acids and non-saponifiable lipids occurs in yeast extracts only in the presence of the soluble supernatant plus a small particle fraction[1,2]. This particulate fraction, sedimentable in 60 min between 25000 × *g* and 60000 × *g*, has been shown to be involved in several different stages of lipogenesis[3,4]. In an effort to determine the cytological origin of these particles, fractionation of the sedimented material has been attempted. In particular, it seemed of interest to learn whether the active material is associated with the membranous portion of the endoplasmic reticulum[5], as has been suggested for stimulatory particles in mammalian lipogenic systems[6].

Cells of *Saccharomyces cerevisiae*, grown anaerobically and then aerated for 2.5 h (ref. 2), were washed in 0.1 M phosphate buffer (pH 7) and resuspended in this buffer to give a suspension containing 1 g (wet wt.) of cells per 2 ml of suspension, after which they were broken in a French pressure cell[7] at 5–6 tons pressure.

Under these conditions, the breakage of cells is of the order of 60–75 %. The resultant homogenate was centrifuged as previously described to obtain the mitochondria-free supernatant[3]. 10 ml of this preparation was centrifuged at 100000 × *g* for 90 min in order to obtain the soluble supernatant and the small particle pellet. The latter was composed of three layers, which could be separated. As can be seen from Table I, the middle fraction (the "red" layer) of particles was most active in stimulating lipid formation. The uppermost fraction, which appeared as a light, fluffy layer, was inactive. This layer was relatively rich in protein and lipids, and is presumed to contain membranous material. The most readily sedimented particles (the "clear" layer) were also rich in protein and contained glycogen (as judged by their reaction with iodine). While this fraction occasionally stimulated lipid formation (probably because of contamination with the "red" layer), its activity on a protein basis was always well below that of the middle layer.

The "red" layer, which accounted for 50–60 % of the total protein, and 80–90 % of the total RNA, of the crude small particle fraction was found to consist of approx.

TABLE I

EFFECT OF VARIOUS SMALL PARTICLE FRACTIONS ON ACETATE INCORPORATION INTO LIPIDS IN THE PRESENCE OF YEAST SUPERNATANT

1 ml soluble supernatant (22 mg protein) made up in a 0.1 M phosphate buffer (pH 7) was incubated in air at 30° on a shaker with 5 μM ATP and 3 μM sodium acetate ($1.8 \cdot 10^6$ counts/min total). Enough particles were added, where shown, to give about 5 mg protein; after 2 h, the reaction was stopped, and lipids were extracted and counted as described previously[3], total volume per flask, 1.5 ml.

Particle fraction added	*Radioactivity incorporated*		*Composition of particles*		
	NSF (counts/min)*	*FAF* (counts/min)*	*Protein (mg/ml)*	*RNA (mg/ml)*	*Lipid (mg/ml)*
None	10 000	17 400			
"Fluffy" layer	12 600	20 200	15.7	1.1	1.2
"Red" layer	75 200	171 600	33.7	17.6	0.2
"Clear" layer	18 400	28 500	17.7	6.0	—

* Abbreviations: NSF, non-saponifiable lipids; FAF, fatty acids.

65 % protein and 35 % RNA, together with a trace of lipid. Since the composition of this "red" layer closely approaches that reported for purified yeast ribosomes[8], it seemed reasonable to attempt to substitute a yeast ribosome preparation for the active particle fraction in our system. For this purpose, a modification of the procedure of MORGAN[9] was followed, since he had reported the preparation of purified 80-S ribosomes from our strain of yeast[10]. As can be seen in Fig. 1, the yeast ribosome preparation and the "red" layer of particles were equivalent on a protein basis in stimulating lipid formation over a wide range of particle concentrations. In this regard, it should be mentioned also that centrifugation, using the Spinco Model E instrument, showed a single major peak, accounting for at least 80 % of the sedimentable material, in each type of preparation used in Fig. 1. Furthermore, there were no significant differences in the minor peaks and the sedimentation patterns of the two preparations.

It thus appears that the active material involved in lipogenesis in yeast is sedimented with the ribosomal fraction of the cells. That this activity is not directly mediated by intact ribosomes can be concluded from earlier work[3] in which it was shown that RNAase (EC 2.7.7.16) treatment did not limit the activity of the small-particle fraction; nor did various treatments asserted to destroy ribosomal structure. The results suggest the presence of particles other than ribosomes in the "ribosomal fraction". Alternatively, it may be possible that enzymes involved in lipid synthesis are present on the ribosomes and that the bound enzymes protect these particular ribosomes from deleterious agents.

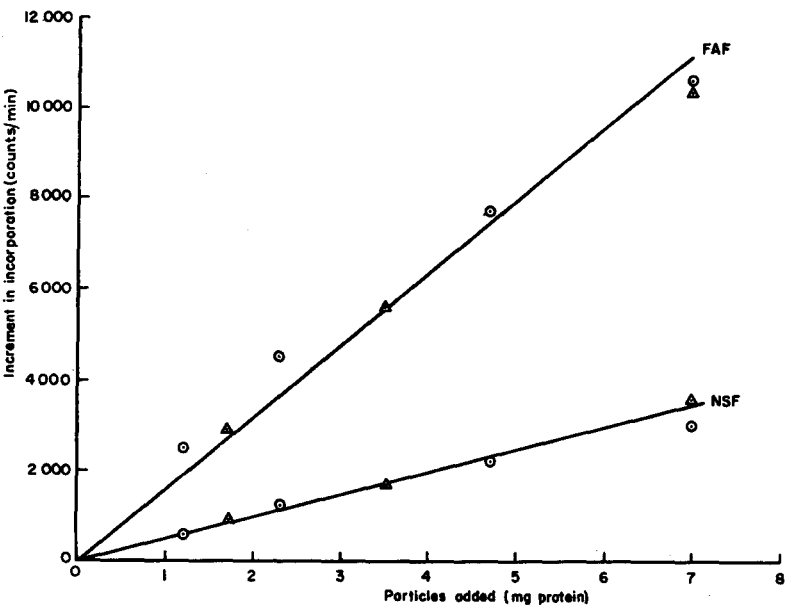

Fig. 1. Comparison of ribosomal preparation and "red" layer in stimulation of lipid synthesis by yeast extract. Cells were grown, harvested and broken as described in the text, except that the final suspending medium consisted of 1 mM Tris buffer (pH 7.5) and 1 mM $MgCl_2$. The resultant homogenate was divided into two portions; one was centrifuged as usual[3] to obtain the "red" layer, the other carried through MORGAN's procedure[9]. The particles were resuspended in the same buffer for use in incorporation studies. The latter as in Table I, except that 0.1 ml of 1 M phosphate buffer (pH 6.3) was added to all tubes and the total radioactive acetate used per tube contained $5.4 \cdot 10^5$ counts/min. Counts incorporated in absence of particles: Non-saponifiable lipids, 800; fatty acids, 7260. ⊙, added ribosomal preparation; △, added "red" layer.

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PN 1294

Inhibition of citrate formation by long-chain acyl thioesters of coenzyme A as a possible control mechanism in fatty acid biosynthesis

Various workers[1–3] have reported that the carboxylation of acetyl-coenzyme A, yielding malonyl-CoA, is the rate-limiting step in fatty acid synthesis. Citrate or isocitrate are required for this synthesis in liver extracts[2,4], and it has now been shown by LYNEN and coworkers[5] that these, or related acids, are absolutely required by rat-liver acetyl-CoA carboxylase (acetyl-CoA:CO_2 ligase, EC 6.4.1.2). The activity of the carboxylase from other tissues is also stimulated[6]. Thus citrate and isocitrate may play a vital role in the regulation of fatty acid synthesis.

Lipogenesis is impaired in livers from fasted or diabetic rats, in which, although oxaloacetate levels are normal[7,8] and acetyl-CoA may be elevated[9], citrate is much diminished in amount[10], suggesting that the activity of citrate synthase (citrate oxaloacetate-lyase (CoA-acetylating), EC 4.1.3.7, formerly known as citrate condensing enzyme) may be reduced. Liver contains much less of this enzyme than does, for example, heart muscle[11].

It has been found[12] that livers from normally fed rats contain 30–60 mμmoles of long-chain acyl-CoA per gram wet weight, but that fasting increases this to 80–180 mμmoles/g. Refeeding fasted animals with fat causes a further increase, while sugar refeeding causes a rapid drop to values below the controls. Fig. 1 shows that palmitoyl-CoA, in a concentration range comparable to these tissue levels of long-chain acyl-CoA, is a profound inhibitor of citrate synthase. Free coenzyme A and palmitate do not cause this inhibition, which is not relieved by an increase in acetyl-CoA concentration. It thus appears possible that the increase in long-chain acyl-CoA which occurs in some conditions may, by reducing citrate formation, result in the inhibition of acetyl-