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Prediction of growth with kinetics of cell-free translation systems

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Summary. The kinetics of cell-free translation systems containing as main components washed rat liver polysomes and muscle cell sap from a muscle piece of the proband, can be used for predicting body growth rate or muscle tissue growth in cattle and swine. A muscle biopsy from the hindquarters of animals aged about 45 days is the source of the muscle fraction. It introduces into the translation systems the required aminoacylated tRNA and translational initiating and elongating factors containing the muscle cell sap fraction of the proband. Based on a polynomial regression of the type

$$Y = b_0 + b \sum_{i=1}^{k} \sum_{n=1}^{3} T_i^n + \underline{e}$$

the coefficient of the multiple correlation between either the growth rate or the lifetime-related muscle mass (Y) of the carcass of boars aged 195 ± 19 days or of bulls aged 450 ± 20 days and the translational kinetics (T) is 0.60–0.8. A generalized expression of the relationship between growth criterions Y (muscle tissue growth rate or nutritional energy expenditure for the growth rate) is

$$s_y^2 = L\, s_e^2 / f(T)$$

where s_y^2 is the variance of the growth criterion; L is a constant, its value is approximately 2; and f(T) is the function mentioned above. If T is measured in sires and Y in male offsprings, the coefficient of the genetic correlation between a linear combination of T_i and Y is 0.6–0.9.

Key words: Growth – Translation – Protein metabolism – Prediction

Introduction

In biology, there are only a few possibilities for quantitatively predicting baselined events, including those of cellular and tissue growth. On the site, the detection of quantitative relationships is a question of error of the estimation of the parameter predicted, but this is generally true for parameters of a biological, physical or chemical variable. However, the validity of a postulated relationship is decided by the predictability of dependents on the independent variable. At present, this criterion is used for determining the relation of ontogenetic or offspring growth in vivo and the translational rate measured in vitro in a early postnatal lifetime of the parents, or individually. Variability is the fundamental feature; it is used to select mathematical statistical procedures for detecting the dependence of growth from the level of translation.

It is known that translational rate and protein biosynthesis are closely correlated (Revel and Groner 1978). There is also a close qualitative correlation between protein synthesis and cellular growth (Pardee et al. 1978). However, no information exists on the quantitative relationship between translation in vitro and body or tissue growth in vivo. Such a relationship must be expected if the limited steps of translation measured at molecular level (Juarez et al. 1975; Carpousis et al. 1977; Messenguy and Delforge 1976; Le Meur et al. 1976) determine tissue growth. In this article, these limited steps have been examined at the level of isoacceptor aminoacyl tRNA supply and at the level of translational initiation capacity. The activity of the intracellular proteolysis was also measured. The conditions of a cell-free translation system can be chosen in a way that the three levels are measurable indirectly.

Methods

Prediction of growth is especially desirable in breeding farm animals. Therefore, investigations were made in cattle and swine. A muscle piece (0.5–1 g *Musculus semimembranosus* of bulls or from the *Musculus glutaeus* region of pigs) was excised from animals aged about 45 days. After homogenization (1:3 w/v) in buffer (50 mM Tris · HCl, pH 7.6, 0 °C; 6 mM $MgCl_2$; 1 mM EDTA · Na_2; 6 mM 2-mercaptoethanol; 250 mM RNase-free saccharose), centrifugation (30,000 × g for 20 min), and Sephadex G 25 filtration of the supernatant, glycerin was added to a final concentration of 25% and the protein content of the muscle sap fraction was determined (Bradford 1976). Aliquotes containing 20, 40 and 80 µg protein were introduced into a cell-free translation system containing 8 and 10–15 µg washed rat liver polysomes in a final volume of 50 and 100 µl, respectively. Two or three incubation times were chosen. More details on the translation system can be found elsewhere (Loehrke 1983).

For polysome isolation, male Wistar rats with a body weight of about 150 g were starved for 16 h, and sacrificed by cervical dislocation. All subsequent procedures were performed at 0–4 °C. Livers were removed immediately, rinsed and weighed in buffer I (50 mM Tris · HCl, pH 7.5; 25 mM KCl; 5 mM 2-mercaptoethanol), then homogenized in 2 volumes of buffer II (250 mM sucrose; 50 mM Tris · Ac, pH 7.5; 30 mM KAc; 5 mM $Mg(Ac)_2$; 2 mM 2-mercaptoethanol) with two strokes using a well-fitted Teflon-glass homogenizer. The homogenate was centrifuged (12,000 g, 20 min). After the removal of lipids by filtration, centrifugation was repeated. The supernatant was mixed with 0.11 Vol 20% Triton X 100 containing 0.5 M $Mg(Ac)_2$; 1 M KCl; 1.5 M NH_4Cl. Thereafter, 0.05 volumes of 10% Nadesoxycholate was added. The mixture was layered over 20% sucrose containing 50 mM Tris · HCl, pH 7.5; 70 mM KCl; 50 mM $Mg(Ac)_2$; 2 mM 2-mercaptoethanol. After ultracentrifugation (90 min, 105,000 × g) the brown membranes over the pellet were removed and the pellet was suspended gently for 40 min in buffer III (250 mM sucrose; 50 mM Tris · Ac, pH 7.5; 400 mM KAc; 50 mM $Mg(Ac)_2$; 5 mM 2-mercaptoethanol; 25% glycerol). Following centrifugation (2 min, 1,500 g), the supernatant was applied to column (1.5 × 20 cm gel bed of Sepharose 2B). The gel was equilibrated and eluted with buffer III without sucrose. Polysomes were visible as a cloudy white fraction. Purified polysomes retained activity when stored in liquid nitrogen for at least 12 weeks.

The incorporation rates of ^{3}H-Leucine (translation rate T) were combined in a regression equation of the type

$$Y = f(T) + \underline{e} = b_0 + b \sum_{i=1}^{k} \sum_{n=1}^{3} T_i^n + \underline{e}$$

where the dependent variable Y is the body weight or muscle mass of the carcass of boars slaughtered at the age of about 195 days and the body weight or muscle mass of the carcass of bulls slaughtered at the age of about 450 days, divided by lifetime days or the nutritional energy expenditure for the growth rate; b represents the partial regression coefficients and *e* is the residual variance. For the estimation of muscle tissue growth the mass of lean carcass parts was divided by lifetime (days). After *t*-testing of b, the regression equation was restricted for non-significant independent variables. The three growth criterions are also measured in offsprings (108 boars being descended from 18 sires, per sire 6 sows) and the independent variable T in 18 sires (T_V). If $\bar{Y}$ is the mean of an offspring group, G_Y is the estimated genotype for the growth criterions, and r_{G_Y,T_V} is the genetic correlation between the estimated genotypic effect for the growth criterions measured in offsprings and translation criterion of the sires, respectively, then

$$r_{G_Y,T_V} = r_{T_V,\bar{Y}}\, 2D/h_T h_Y$$

where

$$D^2 = \frac{4 + h_{\bar{Y}}^2 (N-1)}{4N},$$

h^2 = coefficient of heritability,
N = number of offsprings.

As 7 and 11 offspring groups in two series were proven, it was necessary to compute the mean squares within series and then to combine the data for estimating the genetic correlation.

Results and discussion

The ontogenetic variance of myogenesis estimated by lifetime-divided muscle mass of the carcass of bulls aged about 450 days or of the carcass of boars aged about 195 days is related in a magnitude of 40–65% to the variance of translational criterions measured on the basis of muscle cell sap prepared from a muscle piece that was removed from the animal at the age of nearly 45 days.

The ontogenetic studies also showed that the variance of a growth criterion s_y^2 for both cattle and swine is equal to (variance of Y) on the condition that Y is estimated by the translation kinetics T in early postnatal lifetime:

$$s_y^2 = L\, s_e^2 / f(T)$$

where the constant L in present results is about 2. Then $\frac{1}{2} \cdot s_y$ (Fig. 1) approximates the residual variance *e* of the regression equation $Y = f(T) + e$. This equation based on variance terms has a general character. But the partial regression coefficients vary with varying environmental conditions. In Fig. 1 the real growth values are plotted against predicted values. The b-estimations for the restricted regression equation, the multiple coefficients of correlation R before and after retriction, and the t-values (whereby the theoretical t-value is 1.74) can be seen for a given environment in the legend to Fig. 1.

Genes connected with the variance of translation are closely correlated with muscle growth (Fig. 2). If T is measured in sires and Y in male offspring (pigs), it is not necessary to use the polynomial regression equation because the T of sires and Y of the offspring are related in almost a linear manner (Figs. 2–4). Figures 2–4 show the real mean values and, as multiple regression, the relation between a linear combination of the T-kinetics and Y within one of the series studied and

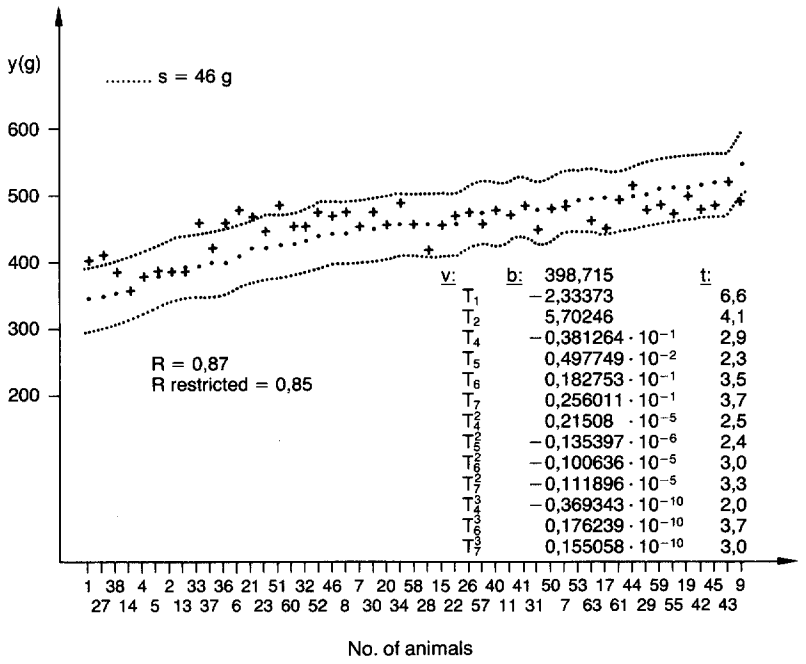

Fig. 1. Ontogenetical real muscle tissue growth against predicted growth (dependent variable y in grams) of pigs (v = independent variable of the polynomial regression equation; b = partial coefficients of regression; t = computed t-values of Students' t-test where theoretical t-value is 1.74); ● real muscle tissue growth; + predicted muscle tissue growth

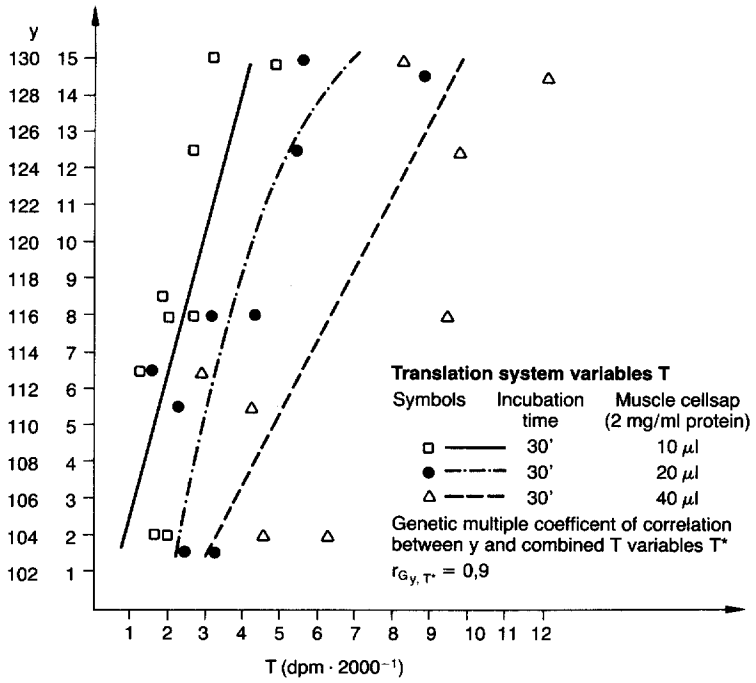

Fig. 2. Muscle tissue growth (y in grams) of pigs (offspring group mean) versus translation rate T of sires (dpm) for the kinetic variant of varying protein concentration but constant reaction time

the estimation of the coefficient of multiple correlation over both series. The physiological interpretation of the genetic relationship is unequivocal. More efficient translation is followed in both faster growth and degressed nutritional energy expenditure per growth rate unit.

Together with the generalizing equation based on variance terms these results demonstrate the quantitative relationship between translation measured in vitro in an early postnatal lifetime and the posterior growth of muscle tissue in vivo in the ontogenesis or in respect to the offsprings.

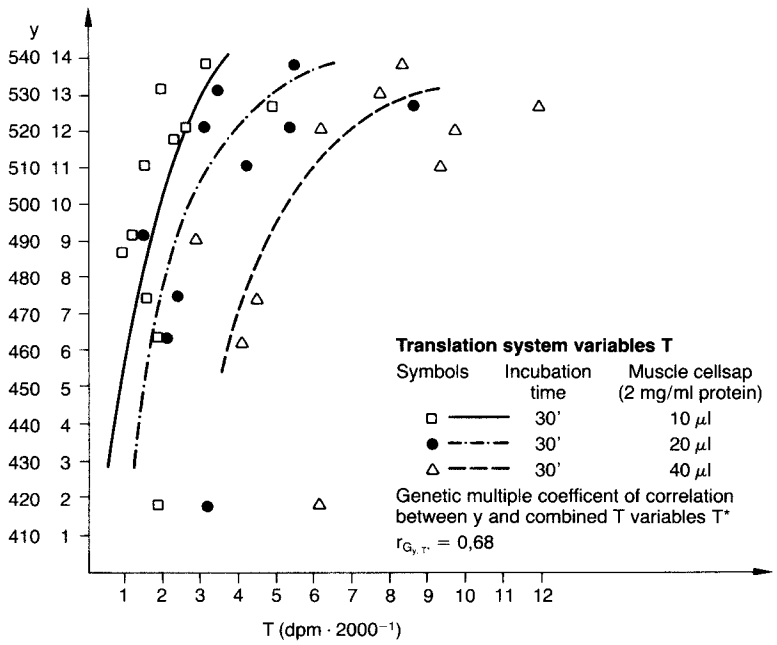

Fig. 3. Growth rate (y in grams) of pigs (body weight of offspring group mean divided by lifetime in days) versus translation rate of sires T (dpm) for the kinetic variant of varying protein concentration but constant reaction time

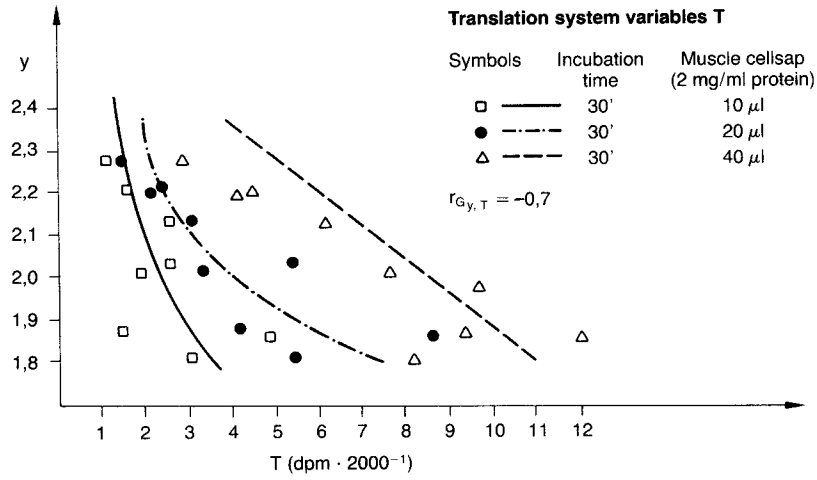

Fig. 4. Nutritional energy expenditure per growth rate (y) of pigs (offspring group mean) versus translation rate of sires T (dpm) for the kinetic variant of varying protein concentration but constant reaction time

References

Revel M, Groner Y (1978) Post transcriptional and translational controls of gene expression in eukaryotes. Annu Rev Biochem 47:1079–1126

Pardee A, Dubrow R, Hamlin J, Kletzien R (1978) Animal cell cyclus. Annu Rev Biochem 47:715–750

Juarez H, Juarez D, Hedgcoth C (1975) Amounts of isoaccepting lysine tRNAs change with the proliferative state of cells. Nature 254:359–360

Carpousis A, Christner P, Rosenbloom L (1977) Preferential usage of tRNS isoaccepting species in collagen synthesis. J Biol Chem 252:8023–8026

Messenguy F, Delforge J (1976) Role of transfer ribonucleic acids in the regulation of several biosyntheses in *Saccharomyces cerevisiae*. Eur J Biochem 67:335–339

Le Meur M, Gerlinger P, Ebel J (1976) Messenger RNA translation in the presence of homologous and heterologous tRNS. Eur J Biochem 67:519–526

Bradford M (1976) Protein assay by dye binding. Anal Biochem 72:248

Loehrke B (1983) Wachstumleistungsfrüherkennung mit Hilfe zellfreier Translationssysteme. Dissertation zur Promotion B. Forschungszentrum für Tierproduktion Dummerstorf-Rostock der Akademie der Landwirtschaftswissenschaften der DDR