Secretory proteins compete for production in the mammary gland of transgenic mice

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To explore the possibility that genes might compete for expression, we have studied transgenic mice producing high levels of the sheep milk protein, β -lactoglobulin (BLG), in the mammary gland. Mice carrying one or more transgene loci expressed BLG in milk at levels ranging from 7 to 33 mg/ml. The effects of BLG synthesis on the levels of endogenous milk gene expression were examined. No significant increase in total milk protein concentration was recorded even in mice expressing the largest amounts of BLG. Measurement of individual milk proteins showed that transgene protein was manufactured at the expense

of host protein synthesized in the gland. Whey acidic protein production was more suppressed than casein production. Suppression of endogenous proteins was matched by a reduction in the corresponding steady-state mRNA levels; in double-transgenic mice, which expressed the largest amounts of BLG, β -casein and whey acidic protein mRNA populations were reduced to 75 and 56% of control levels respectively. We demonstrate that an exogenous gene competes effectively for expression with endogenous genes. Possible mechanisms of competition are discussed.

INTRODUCTION

The functional expression of gene products is controlled at a variety of transcriptional and post-transcriptional levels. Most analyses to date have made the assumption that expression of an individual gene product is essentially independent of the expression of others. However, the question of competition for expression has not been fully addressed. Indeed, there are many cases where competition might be expected to operate. In lymphocytes, for example, heavy- and light-chain immunoglobulin gene expression depends on a common pool of transcription factors [1,2], and transcripts are processed and translated through the same pathways. In erythrocytes, the co-ordinated expression of α - and β -globin transcripts and their encoded proteins is critical for the regulated manufacture of haemoglobin [3–5].

Despite these and other equally obvious examples where competition might be expected to play a role, only one study has explored the possibility that such competition may exist in mammalian cells. In pioneering experiments, Davis and MacDonald [6] used transgenic mice expressing rat elastase to examine effects of introduced genes on endogenous genes; while they failed to detect changes in the rates of endogenous mouse elastase gene transcription, mouse elastase mRNA levels appeared to decline as transgene mRNA levels increased.

In the present report we investigate gene expression competition in the quite different system of the mammary gland where, during lactation, the epithelial tissue produces very high levels of milk protein. Transgenic mice carrying the gene for the sheep milk protein β -lactoglobulin (BLG) have been used to study the effects of an introduced gene on the manufacture of mouse milk proteins. Lines of transgenic mice that secrete BLG into their milk have been described previously [7]. BLG is expressed at high levels in the mammary gland and is developmentally regulated in much the same way as endogenous β -casein in milk [8]. Although BLG is the major whey protein produced by the sheep mammary gland, and is part of normal milk composition in ruminants and other species, no equivalent

protein homologue is present in mouse milk. In one line of transgenic mice expressing substantial amounts of BLG we have shown that mammary development, carbohydrate and fat metabolism and overall milk yield are unaffected [9]. We have now produced mice that express significantly higher levels of BLG by crossing existing transgenic lines or by breeding a line to homozygosity. We have used these mice to evaluate the effect of transgene expression on endogenous gene expression and we report that significant competition occurs.

MATERIALS AND METHODS

Animals

Transgenic mouse lines 7 and 45 carry multiple copies of the gene for the sheep milk protein BLG [10]. In both lines the copies are integrated in a tandem array at single sites. The derivation of these lines has been described previously [7]. Line 7 carries 25 copies of a 16.2 kb *SalI* fragment, whereas line 45 carries 17 copies of a shorter 10.5 kb *SalI*–*XbaI* fragment [11]. Both fragments contain the BLG transcription unit (4.9 kb) with 4 kb of 5'-flanking sequences and either 7.3 kb (line 7) or 1.6 kb (line 45) of 3'-flanking sequences. All mice used were of CBA/C57 F₂ hybrid background. Mice from lines 7 and 45 were crossed to generate single- and double-transgenic animals; non-transgenic, 7, 45 and 7 × 45 females were identified by Southern blotting of tail DNA samples [12]. A further group of double-transgenic females was generated by breeding homozygous 45 females, using a homozygous 45 colony confirmed by progeny testing.

Sample collection

Naturally impregnated mice were restricted to five suckling pups on delivery. At 11 days post-parturition, milk was collected [7] and dispensed into 50 μ l amounts using a Hamilton syringe. Aliquots were weighed to check the measurement accuracy and stored at –20 °C. Milk pools were constructed using animals of

the same genotype (non-transgenic pool, $n = 13$; 7 pool, $n = 16$; 45 pool, $n = 13$; 7 $\times$ 45 pool, $n = 16$; 45 $\times$ 45 pool, $n = 12$). Mice were killed after milking, when mammary tissue samples were collected and stored in liquid nitrogen.

Protein analysis

The protein contents of milk samples were measured by the Kjeldahl method [13], adapted for small volume analysis. Briefly, milk was diluted 1:4 (v/v) in distilled water and the protein precipitated with trichloroacetic acid [10% (w/v) final concentration]. Protein pellets were washed with acetone, dissolved in 2 M NaOH and boiled in concentrated H_2SO_4 in the presence of catalysts (K_2SO_4 , Cu_2SO_4 , $Na_2SeO_4 \cdot 10H_2O$). Resulting NH_4^+ was converted into NH_3 by adding 10 M NaOH and collected by distillation into boric acid indicator. The amount of nitrogen in each distillate was calculated by titration against calibrated HCl, and values were multiplied by 6.38 to give protein concentrations.

SDS/PAGE analysis of milk proteins was performed using 15% or 17.5% discontinuous gels of 0.75 mm thickness. Defatted milk samples were further diluted 1:4 (v/v) in reducing loading buffer, boiled and sample volumes readjusted before loading on to gels. After electrophoresis, gels were stained with Coomassie Blue G-250, destained and scanned at 585 nm using a Shimadzu CS-9000 densitometer. Preliminary experiments were carried out to ascertain that individual proteins were adequately resolved for measurement by scanning densitometry, and that measurements fell within the respective linear absorbance ranges for the dye-binding capacities of the proteins under examination.

Sheep BLG was purified from fresh pooled milk [14], checked for purity by gel and Western-blot analysis, and the protein content determined by the micro-Kjeldahl technique.

RNA analysis

Mice were killed at 11 days post-parturition and total RNA was isolated from mammary-gland tissue using RNazol [15] according to the manufacturer's specifications (Cinna/Biotex Laboratories, Houston, TX, U.S.A.). Northern blots were performed on 10 μ g of total RNA electrophoresed in 1.5% Mops/agarose gels which were stained with ethidium bromide, before transfer in 20 $\times$ SSC to Hybond N membranes (where 1 $\times$ SSC is 0.15 M NaCl/0.015 M sodium citrate). Blots were probed using the method of Church and Gilbert [16] with radiolabelled probes for sheep BLG (424 bp *Pst*I cDNA fragment [8]), mouse whey acidic protein (WAP) (380 bp *Eco*RI fragment from pSP51-9 [8]) and mouse β -casein (insert from pCmb13 [17]). For dot-blotting, total RNA was heated to 65 $^\circ$ C and 1 μ g samples in 2.2 M formaldehyde were loaded on to Hybond N membranes pre-wetted with distilled water, using a 96-well manifold (BRL). Separate hybridizations were carried out using sheep BLG, mouse WAP, mouse β -casein and *Xenopus* rDNA [18] probes. After washing, the filters were exposed to X-ray film. Individual dots on the filter were excised and counted in a scintillation counter. Triplicate samples for each RNA preparation were counted and corrected for loading by reference to hybridization reactions with the rDNA probe.

RESULTS

To examine whether milk protein genes compete for expression during lactation, milk samples from mice transgenic for the sheep whey protein BLG were analysed at both RNA and protein levels. Mouse lines BLG7 and BLG45 harbour tandem copies of the BLG gene integrated at a single locus and transmitted in a stable Mendelian fashion. Double hemizygous

Table 1 Measurement of total milk protein and BLG in control and transgenic mouse milk pools

The total protein contents of milk samples were measured by the micro-Kjeldahl technique, and BLG concentrations in milk were calculated by scanning densitometry of Coomassie Blue-stained polyacrylamide gels, as described in the Materials and methods section. Values are means $\pm$ S.E.M. for the number of assays indicated in parentheses.

Mouse milk pool	BLG concentration (mg/ml)	Total milk protein concentration (mg/ml)
Non-transgenic control	—	76.7 $\pm$ 0.5 (6)
Line 7	7.4 $\pm$ 1.0 (4)	84.9 $\pm$ 2.0 (4)
Line 45	18.9 $\pm$ 0.8 (4)	75.3 $\pm$ 1.8 (4)
7 $\times$ 45	27.9 $\pm$ 1.6 (5)	79.7 $\pm$ 2.3 (4)
45 $\times$ 45	33.2 $\pm$ 0.8 (8)	78.4 $\pm$ 1.7 (3)

(7 $\times$ 45) and homozygous (45 $\times$ 45) transgenic female mice were generated and used to investigate the effects of transgene dosage on milk protein composition.

BLG transgene expression increases with transgene locus dose

BLG levels were measured by scanning densitometry of Coomassie Blue-stained SDS/polyacrylamide gels of pooled milk samples from single (7 and 45) and double (7 $\times$ 45 and 45 $\times$ 45) transgenic mice (Table 1). Known amounts of BLG purified from sheep milk were used for calibration.

Mice from both lines 7 and 45 produced significant amounts of BLG in their milk. Line 45 mice expressed BLG at an average level of 19 mg/ml. However, the average amount of BLG in line 7 mice was 7 mg/ml, lower than previously reported. Earlier data indicated that line 7 mice synthesize BLG at levels similar to line 45 mice [7]. Gel analyses of individual milks that formed the line 7 milk pool revealed a range of transgene protein expression. The transgene copy number appeared to be stable in these animals, and a subsequent study of 35 individual line 7 mice showed that BLG concentrations can vary from 3 to 18 mg/ml, suggesting an insertion site-specific influence on the stability of transgene expression (K. Dobie and M. McClenaghan, unpublished work).

A clear additive effect was observed, however, when BLG levels in these mice were compared with levels in mice with two transgene arrays (Table 1). Milk BLG concentration measured in 7 $\times$ 45 mice was 27.9 mg/ml, significantly higher than the mean levels in either line 7 (7.4 mg/ml) or line 45 mice (18.9 mg/ml). The greatest concentration of BLG in milk (33.2 mg/ml) was recorded in 45 $\times$ 45 homozygous mice, approaching twice the amount found in hemizygous mice.

BLG expression has little effect on overall milk protein concentration

The use of double-hemizygous and homozygous transgenic mice provided milk with very high concentrations of BLG. To determine the effect of such high level transgene expression on mammary-gland protein production, the protein concentrations of milk pools were assayed by a micro-Kjeldahl technique (Table 1). There was no correlation between transgene protein synthesis and milk protein concentration; indeed the three milk pools with the highest concentrations of BLG (45, 7 $\times$ 45 and 45 $\times$ 45 pools) showed no increase in milk protein content when compared with non-transgenic mouse milk. A small increase in milk protein was nevertheless recorded in line 7 mice which, on average, produced the lowest levels of BLG.

Figure 1 SDS/PAGE analysis of milk proteins from transgenic and non-transgenic mice

Samples of milk (0.16 μ l) were electrophoresed in 15% polyacrylamide gels (1.5 mm thick) under reducing conditions and stained with Coomassie Blue. (a) The lanes were as follows: BLG, 1 μ g of ovine BLG; CON, control non-transgenic mouse milk pool; 7, line 7 milk pool; 45, line 45 milk pool; 7 $\times$ 45, 7 $\times$ 45 milk pool; 45 $\times$ 45, 45 $\times$ 45 milk pool; LMM, low-molecular-mass standards, (phosphorylase *b*, 94 kDa, albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; trypsin inhibitor, 21 kDa). Individual milk proteins are indicated. (b) and (c) Milk profiles from five individual non-transgenic control mice and five individual 45 $\times$ 45 transgenic mice respectively; milk from these mice contributed to the experimental milk pools. Levels of the different milk proteins in these samples were compared using scanning densitometry. Albumin, caseins, WAP and BLG levels showed low variability in both control ($n = 5$) and 45 $\times$ 45 ($n = 7$) mice; the standard error was less than 5.25% for each protein assayed.

BLG expression reduces the concentration of endogenous milk proteins

The observation that total milk protein production does not vary significantly despite massive expression of the transgene shows that the expression of BLG suppresses the production of one or more endogenous mouse milk proteins. To address this, individual milk proteins in the pool samples were separated by SDS/PAGE, stained (Figure 1) and quantified by scanning densitometry. The proteins selected for measurement were α - and β -caseins, a partially characterized 25.1 kDa casein [19], WAP and serum albumin.

The levels of the three endogenous caseins (α -, β - and 25.1 kDa) decreased by similar amounts as the concentration of BLG increased (Figure 1a and Table 2). The casein levels in milk from line 7 mice, which expressed on average the lowest amounts of BLG, were 83–84% of control levels. Milks with an intermediate amount of BLG (45 and 7 $\times$ 45 mice) had casein concentrations representing 62–65% of controls. Milk from 45 $\times$ 45 mice which expressed the most BLG had the lowest concentration of each casein, equivalent to 50–55% of control levels. The concentration of the major whey protein, WAP, also decreased as BLG levels increased. WAP concentrations appeared to be even more affected than the caseins by the expression of BLG; for example, WAP levels in milk from 45 $\times$ 45 mice were reduced to 35% of

control values. Measurement of caseins, WAP and albumin in milk samples from control and 45 $\times$ 45 mice showed that there was little variation in milk composition among individuals of the same genotype (Figures 1b and 1c).

Serum albumin, unlike casein and WAP proteins, is not expressed in the secretory epithelial tissue of the mammary gland (M. Shani, personal communication) but is derived from blood. In contrast with the other endogenous proteins, there was no significant alteration in the concentration of albumin irrespective of the level of BLG produced (Table 2).

BLG expression suppresses endogenous milk protein mRNAs

To determine whether the transgene and endogenous milk protein levels correlated with their respective steady-state mRNA populations, total RNA preparations were isolated from mammary glands of lactating control, 45, 7 $\times$ 45 and 45 $\times$ 45 females and analysed by Northern and dot blotting followed by hybridization. Gene probes for mouse β -casein, WAP and BLG were used to probe Northern blots of total RNA and the relative steady-state levels of the various mRNAs were quantified using dot blots.

As expected, no BLG signal was detected in non-transgenic controls whereas abundant approx. 800 bp transcripts were present in all the transgenic samples. BLG mRNA levels increased

Table 2 Protein composition of control and transgenic mouse milk pools

The protein profiles of each mouse milk pool (non-transgenic, 7, 45, 7 × 45 and 45 × 45) were compared within the same Coomassie Blue-stained polyacrylamide gel and individual proteins were measured by scanning densitometry (see the Materials and methods section). The levels of proteins in transgenic mouse milk pools are expressed as percentages of the amounts recorded in the control mouse milk pool and are means ± S.E.M. for the number of measurements indicated in parentheses. The amounts of BLG present in milk samples are given as a percentage of total milk protein.

Milk pool	α -Casein	β -Casein	25.1 kDa Casein	WAP	Albumin	BLG
Control	100	100	100	100	100	—
7	84.2 ± 6.4 (5)	83.3 ± 4.6 (5)	83.0 ± 4.4 (5)	65.9 ± 7.2 (4)	92.3 ± 5.2 (3)	8.72
45	66.1 ± 2.6 (5)	65.3 ± 1.1 (5)	62.1 ± 0.8 (5)	55.7 ± 7.3 (4)	91.7 ± 1.7 (4)	25.1
7 × 45	66.1 ± 4.6 (3)	61.8 ± 1.3 (3)	61.7 ± 1.6 (3)	67.1 ± 1.5 (2)	100.3 ± 4.4 (2)	35.0
45 × 45	50.5 ± 1.9 (8)	53.3 ± 2.3 (8)	54.4 ± 1.8 (8)	34.5 ± 2.6 (8)	99.9 ± 3.1 (5)	42.3

Table 3 Dot-blot analysis of RNA from mid-lactation mammary-gland tissue from control and transgenic mice

Dot-blots of total RNA samples (1 µg loadings) were hybridized to BLG, β -casein and WAP DNA probes. After washing, the degree of hybridization was measured by scintillation counting. Six individuals per group were examined and data analysed by Student's *t*-test. mRNA levels for β -casein and WAP were significantly lower than control values in 7 × 45 mice ($P < 0.01$, $P < 0.05$ respectively) and in 45 × 45 mice ($P < 0.001$). The steady-state levels of endogenous milk protein mRNAs are presented as percentages of the controls and are means ± S.E.M. The levels of BLG mRNA are expressed relative to hybridization measured in hemizygous line 45 samples (means ± S.E.M.).

Milk pool	β -Casein	WAP	BLG
Control	100 ± 2.7	100 ± 8.5	—
45	96 ± 4.5	90 ± 3.1	1.0 ± 0.06
7 × 45	87 ± 2.7	75 ± 3.1	1.27 ± 0.07
45 × 5	74 ± 2.7	56 ± 3.1	1.97 ± 0.16

with the number of transgenic loci and these differences were quantified by dot-blot hybridization (Table 3); for example, the highest levels of BLG mRNA were recorded in homozygous 45 × 45 mice, approximately twice that measured in the hemizygous 45 mice. Similarly, the amount of BLG mRNA in the double transgenic 7 × 45 hemizygous animals was greater than that in individual hemizygous mice. Concomitantly, the steady-state levels for WAP and β -casein mRNAs decreased in 45 and 7 × 45 animals, and homozygous 45 × 45 animals gave the lowest levels. In all three transgenic groups WAP mRNA was suppressed more than β -casein. In 45 × 45 mice, WAP mRNA levels were reduced to 56 % whereas in the same animals β -casein mRNA dropped to only 75 %, relative to control values.

DISCUSSION

Competition among genes for cellular factors has not been fully addressed as a possible mechanism for the mediation of gene expression, polypeptide synthesis and protein export. In this paper we sought to determine by the use of transgenic animals whether gene competition does indeed exist. The effects of foreign milk protein expression on endogenous milk protein production were studied in two lines of transgenic mice expressing high levels of the sheep BLG gene. By using animals that carry one or two transgene loci, we were able to analyse four groups of mice that expressed BLG in milk at concentrations ranging from 7 to 33 mg/ml.

BLG expression did not result in a corresponding increase in the protein content of transgenic mouse milk. Total protein

content was unchanged but the concentrations of the endogenous milk proteins were reduced. The three caseins (α -, β - and 25.1 kDa) and the whey protein (WAP) were all affected. The degree of suppression correlated with the level of BLG expression and was reflected at both the mRNA and protein levels. WAP levels were suppressed more than the caseins. In contrast, the amount of serum albumin (a protein not synthesized in the mammary gland) present in milk was unaffected by BLG expression. No detrimental effects of BLG expression were observed in the lactating females or their pups. It has previously been shown that line 45 mice exhibit normal mammary development and milk yield [9]. These mice also show rates of epithelial cell proliferation comparable with non-transgenic mice during early pregnancy (B. Binas and B. Gusterson, personal communication). Therefore the effects of BLG expression on milk composition appear to be due to competition operating between the transgene and endogenous genes within the secretory epithelial cell.

We find no evidence for the inhibition of transgene expression; this is observed in transgenic plants where co-suppression occurs between homologous genes, resulting in the suppression of both the transgene and endogenous gene [20,21]. This phenomenon is thought to be the result of a homology-dependent interaction and not simply competition resulting from transgene dosage.

In mammals, competition between transgenes and endogenous genes may occur at the transcriptional, mRNA-processing or translational stages in the cell. In mice expressing BLG, suppression of endogenous milk proteins is reflected by the decrease in levels of steady-state mRNA, so it is feasible that competition occurs at the transcriptional level. The genes for β -casein, WAP and BLG are transcribed at relatively high rates (at least 20-fold more than β -actin; A. J. Clark, unpublished work). Such abundantly transcribed genes may compete for a limited pool of a transcription factor/s, such as the recently described STAT protein MPBF (MGF), which is thought to mediate the induction of transcription through prolactin for a variety of milk protein genes [22,23] (STAT, MPBF and MGF are defined as signal transducer and activator of transcription, milk-protein binding factor and mammary-gland binding factor respectively). Different promoters would presumably bind such a factor/s with different affinities thereby explaining the differing degrees of suppression observed. The whey protein WAP, which was more affected than the caseins by BLG competition, has been shown to have a different temporal pattern of expression from β -casein during gestation, suggesting a differential pattern of dependence on available transcription factors [8].

Transcription rates (estimated by nuclear run-ons) for WAP and β -casein genes in control ($n = 6$) and 45 × 45 homozygous ($n = 6$) mice were not significantly different, because of large

intersample variation (results not shown). This is a difficulty encountered when using the technique, and can preclude the detection of relatively modest differences such as those (1.5–2.5 fold) reported in our study. However, effects on rates of transcription have been measured for rat elastase transgenes and the endogenous elastase genes in the exocrine cells of the pancreas [6]. Endogenous elastase mRNA levels were reduced but no change was detected in the transcription rate of the gene, suggesting that competition occurred at a post-transcriptional stage. It has been shown in a number of cell types that steady-state mRNA levels are affected by a range of co- and post-transcriptional processes including mRNA capping, splicing, poly(A) addition and transport from the nucleus to the cytoplasm [24,25]. The regulation of steady-state mRNA levels has also been shown to occur at the level of cytoplasmic turnover. For example, the prolactin-induced accumulation of rat casein mRNA in mammary organ culture is due in part to the increased transcript half-life [26].

Finally, competition may occur at the level of translation, or even at post-translational stages such as docking and entry of the encoded polypeptides into the endoplasmic reticulum, and passage through the secretory pathway. Short-term mammary explant cultures from hemizygous line 45 mice exhibited a significantly higher rate of total protein synthesis than non-transgenic controls, suggesting that a limit to protein synthesis *in vivo* is removed rapidly in culture [9]. Therefore, in addition to the competition at mRNA level demonstrated in the present study, there may be other downstream factors that limit the translational or secretory capacity of the mammary epithelial cell. A link between availability of translational machinery and steady-state mRNA levels has been proposed in other tissues. In a study of the parotid-specific-protein gene in the developing mouse parotid gland, high transcription rates but low mRNA accumulation were observed [27]; the authors suggested that abundance of endoplasmic reticulum determined the mRNA level for this secreted protein. Similarly, Mason et al. [28] proposed that proliferation of secretory apparatus could mediate the stabilization of IgH mRNA in plasma cells.

We have established that competition for gene expression occurs in the mammary gland of transgenic mice and has consequences for milk composition. Further work, employing suitably modified transgenes, will be required to determine the level(s) at which this competition operates.

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REFERENCES

- 1 Singh, H., Sen, R., Baltimore, D. and Sharp, P. A. (1986) *Nature* (London) **319**, 154–158
- 2 Staudt, L. M., Singh, H., Sen, R., Wirth, T., Sharp, P. A. and Baltimore, D. (1986) *Nature* (London) **323**, 640–643
- 3 Dillon, N. and Grosveld, F. (1993) *Trends Genet.* **9**, 134–137
- 4 Felsenfeld, G. (1993) *Gene* **135**, 119–124
- 5 Grosveld, F., Antoniou, M., Berry, M. et al. (1993) *Philos. Trans. R. Soc. Lond. (Biol.)* **339**, 183–191
- 6 Davis, B. P. and MacDonald, R. J. (1988) *Genes Dev.* **2**, 13–22
- 7 Simons, J. P., McClenaghan, M. and Clark, A. J. (1987) *Nature* (London) **328**, 530–532
- 8 Harris, S., McClenaghan, M., Simons, J. P., Ali, S. and Clark, A. J. (1991) *Dev. Genet.* **12**, 299–307
- 9 Wilde, C. J., Clark, A. J., Kerr, M. A., Knight, C. H., McClenaghan, M. and Simons, J. P. (1992) *Biochem. J.* **284**, 717–720
- 10 Ali, S. A. and Clark, A. J. (1988) *J. Mol. Biol.* **199**, 415–426
- 11 Whitelaw, C. B. A., Harris, S., McClenaghan, M., Simons, J. P. and Clark, A. J. (1992) *Biochem. J.* **286**, 31–39
- 12 Whitelaw, C. B. A., Archibald, A. L., Harris, S., McClenaghan, M., Simons, J. P. and Clark, A. J. (1991) *Transgenic Res.* **1**, 3–13
- 13 Walstra, P. and Jenness, R. (1984) in *Dairy Chemistry and Physics*, pp. 384–385, John Wiley and Sons, New York
- 14 Aschaffenburg, R. and Drewry, J. (1957) *Biochem. J.* **65**, 273–277
- 15 Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
- 16 Church, G. M. and Gilbert, W. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1991–1995
- 17 Gupta, P., Rosen, J. M., Eustachio, P. D. and Ruddle, F. H. (1982) *J. Cell Biol.* **93**, 199–204
- 18 Sollner-Webb, B. and Reeder, R. H. (1979) *Cell* **18**, 485–499
- 19 Stevenson, E. M. and Leaver, J. (1994) *Int. Dairy J.* **4**, 205–220
- 20 Jorgensen, R. (1990) *Tibtech* **8**, 340–344
- 21 Flavell, R. B. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3490–3496
- 22 Watson, C. J., Gordon, K. E., Robertson, M. and Clark, A. J. (1991) *Nucleic Acids Res.* **19**, 6603–6610
- 23 Wakeo, H., Gouilleux, F. and Groner, B. (1994) *EMBO J.* **13**, 2182–2192
- 24 Urlaub, G., Mitchell, P. J., Ciudad, C. J. and Chasin, L. A. (1989) *Mol. Cell. Biol.* **9**, 2868–2880
- 25 Atwater, J. A., Wisdom, R. and Verma, I. M. (1990) *Annu. Rev. Gen.* **24**, 519–541
- 26 Guyette, W. A., Matusik, R. J. and Rosen, J. M. (1979) *Cell* **17**, 1013–1023
- 27 Shaw, P., Sordat, B. and Schibler, U. (1986) *Cell* **47**, 107–112
- 28 Mason, J. O., Williams, G. T. and Neuberger, M. S. (1988) *Genes Dev.* **2**, 1003–1011