Hydroxylation of Lysine and Glycosylation of Hydroxylysine During Collagen Biosynthesis in Isolated Chick-Embryo Cartilage Cells

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Hydroxylation of lysine and glycosylation of hydroxylysine during collagen biosynthesis in isolated chick-embryo cartilage cells were studied by using continuous labelling and pulse-chase labelling experiments with [^{14}C]lysine. Control experiments with [^{14}C]proline indicated that in continuous labelling the hydroxylation of [^{14}C]proline became linear with time after about 4 min and the secretion of collagen after about 35 min, as reported previously. In similar experiments with [^{14}C]lysine the hydroxylation of [^{14}C]lysine and the glycosylations of hydroxy[^{14}C]lysine became linear at about 4 min, suggesting that these reactions were initiated while the polypeptide chains were growing on the ribosomes. Pulse-chase labelling experiments with [^{14}C]lysine indicated that after a 5 min pulse-label the hydroxylation of [^{14}C]lysine and the glycosylations of hydroxy[^{14}C]lysine continued during the chase period for about 20 min. The data suggest that these reactions are continued after the release of complete polypeptide chains into the cisternae of the endoplasmic reticulum, whereas the reactions are probably not continued after the formation of the triple helix and the movement of the molecules into the Golgi vacuoles.

The biosynthesis of the collagen molecule involves a number of post-translational modifications of the initial polypeptide chains. Some of these modifications take place before procollagen is secreted from the cells into the extracellular matrix and some of them occur after the secretion. The intracellular modifications consist of hydroxylation of appropriate proline and lysine residues to hydroxyproline and hydroxylysine residues, glycosylation of appropriate hydroxylysine residues to galactosylhydroxylysine and glucosylgalactosylhydroxylysine residues, chain association, disulphide bonding and formation of the triple helix (for reviews on collagen biosynthesis see: Schofield & Prockop, 1973; Bornstein, 1974; Martin *et al.*, 1975; Prockop *et al.*, 1976).

A number of observations have indicated that the hydroxylations of proline and lysine residues are initiated while the polypeptide chains are growing on the ribosomes, but that they are continued after the release of completed polypeptide chains from the ribosomes into the cisternae of the endoplasmic reticulum (see: Uitto & Prockop, 1974; Harwood *et al.*, 1975a; Prockop *et al.*, 1976). In a study on subcellular fractionation of [^{14}C]lysine-labelled chick-embryo fibroblasts Brownell & Veis (1975) demonstrated that the glycosylations of hydroxylysine residues are likewise initiated while the polypeptide chains are still in the stages of assembly on the ribosomes, but it is not known whether they are continued in other compartments of the cell. Assays of collagen galactosyltransferase and collagen glucosyltransferase activities in subcellular fractions of chick-embryo

tendon cells indicated that most of these enzyme activities were associated with the rough-endoplasmic-reticulum fraction, but significant amounts were also present in the smooth endoplasmic reticulum (Harwood *et al.*, 1975b).

Studies on the properties of collagen galactosyltransferase (Risteli *et al.*, 1976) and collagen glucosyltransferase (Myllylä *et al.*, 1975b) have indicated that, although these enzymes catalyse *in vitro* additional glycosylation of hydroxylysine residues in denatured collagens, they do not catalyse the reaction with native collagen. These observations suggest that in the biosynthesis of collagen the glycosylation reactions take place before the triple-helix formation of the polypeptide chains. Other studies suggest that the disulphide bonding (Harwood *et al.*, 1973, 1975a; Uitto & Prockop, 1973, 1974; Schofield *et al.*, 1974) and the triple-helix formation (Olsen *et al.*, 1975; Prockop *et al.*, 1976) occur before the procollagen polypeptide chains pass from the cisternae of the endoplasmic reticulum into the Golgi vacuoles. On the basis of these indirect observations, it seems likely that the glycosylation reaction would not continue within the Golgi vacuoles.

Cartilage cells synthesize type II collagen, which differs from type I collagen of tissues such as bone, skin, tendon and dentine owing to the relatively high extent of hydroxylation of lysine residues and glycosylation of hydroxylysine residues (Miller, 1971). Studies on the biosynthesis of cartilage procollagen have indicated that the disulphide bonding and the triple-helix formation occur considerably later in the

biosynthesis of this collagen than in that of type I collagen in tendon cells (Uitto & Prockop, 1973, 1974; Schofield *et al.*, 1974; Harwood *et al.*, 1975a). Pulse-chase experiments with isolated chick-embryo cartilage cells have indicated that after a 4 min pulse-label a chase period of more than 12 min (Harwood *et al.*, 1975a) or of about 18 min (Uitto & Prockop, 1974) is required for maximal disulphide bonding. The triple-helix formation was found to occur soon thereafter (Uitto & Prockop, 1974). However, the maximal values found in the tests used by Uitto & Prockop (1974) suggest that only about one-half of the intracellular procollagen polypeptide chains became disulphide bonded and triple-helical.

In the present work we studied the time-courses for hydroxylation of lysine residues and glycosylation of hydroxylysine residues in isolated chick-embryo cartilage cells. Special emphasis was laid on the question of whether the glycosylations take place mainly within 15–20 min after a 5 min pulse-label, suggesting that they would occur within the cisternae of the endoplasmic reticulum before the triple-helix formation, or whether they take place mainly after a chase period of more than 15–20 min, suggesting that they occur within the Golgi vacuoles.

Experimental

Materials

Fertilized eggs of white Leghorn chickens were obtained from Siipikarjanhoitajien liitto r.y. (Hämeenlinna, Finland) and they were incubated in a moist atmosphere at 37°C until used.

[¹⁴C]Proline and [¹⁴C]lysine were from New England Nuclear Corp. (Boston, MA, U.S.A.), purified bacterial collagenase was from Sigma Chemical Co. (Kingston-upon-Thames KT2 7BH, U.K.), and trypsin [2.5% (w/v) solution in 0.9% NaCl] was from Gibco Corp. (Grand Island, NY, U.S.A.). Foetal calf serum was from Flow Laboratories (Irvine, Ayrshire, U.K.).

Galactosylhydroxylysine and glucosylgalactosylhydroxylysine were prepared from sponge collagen as described previously (Katzman *et al.*, 1972; Anttinen & Kivirikko, 1976).

Isolation and incubation of matrix-free cells from cartilage

Cells were isolated by enzymic digestion from the sterna of 17-day-old chick embryos by using trypsin and collagenase (Dehm & Prockop, 1973). The cells were filtered through lens paper and then washed three times with modified Krebs medium containing 20% (w/v) foetal calf serum (Dehm & Prockop, 1973). In most experiments 4×10^6 – 8×10^6 cells were

obtained from each sternum. The cells were incubated in modified Krebs medium containing 20% foetal calf serum with moderate shaking at 37°C (Dehm & Prockop, 1973). The radioactive isotopes were added as indicated in various experiments and, after the incorporation periods indicated, 2.0 ml samples of the incubation system (cells plus medium) were quickly pipetted into test tubes containing 0.1 vol. of modified Krebs medium with sufficient cycloheximide and $\alpha\alpha'$ -bipyridyl to provide a final concentration of 100 µg of cycloheximide and 1 µmol of $\alpha\alpha'$ -bipyridyl/ml (Dehm & Prockop, 1971). The samples were centrifuged at 1200g for 10 min at room temperature (23°C) in order to separate the medium from the cells and the cell pellet was washed with 1.5 ml of modified Krebs medium containing 100 µg of cycloheximide and 1 µmol of $\alpha\alpha'$ -bipyridyl/ml. The sample was then re-centrifuged and the wash solution was discarded.

Assays

After incubation with [¹⁴C]proline, the medium and cell fractions were dialysed against running tap-water for 24–48 h and the retained materials were hydrolysed in sealed tubes in 6M-HCl at 120°C overnight. The hydrolysed samples were evaporated on a steam bath and dissolved in 4 ml of water. The hydroxy[¹⁴C]proline content was assayed by a specific radiochemical procedure (Juva & Prockop, 1966).

After incubation with [¹⁴C]lysine, the medium and cell fractions were dialysed against running tap-water as above. The samples were evaporated on a steam bath and hydrolysed with 2.0 ml of 2M-NaOH in sealed disposable glass ampoules at 105°C for 24 h. After hydrolysis the samples were diluted with 4 ml of water and titrated with 2M-HCl to pH 3.

The samples were applied to small columns (1 cm × 3 cm) containing 1 g of Dowex 50 (X8, H⁺ form), and the columns were washed with 7 ml of 0.1 M-sodium citrate/sodium phosphate buffer, pH 3, followed by 10 ml of water (Myllylä *et al.*, 1975a). The columns were then eluted with 7 ml of 1.5M-NH₃. The NH₃ was evaporated from the eluates over a steam bath and the samples were dissolved in 2 ml of the buffer used for amino acid analysis. A small portion was taken for the assay of total radioactivity in the sample and the rest was assayed in the amino acid analyser.

In some experiments a sample treated as described above was divided into three portions. One of them was analysed in the amino acid analyser, whereas the other two were hydrolysed further, one with 6M-HCl at 110°C for 12 h, and the other with 0.2M-HCl at 110°C for 3 h. They were then evaporated and dissolved in the buffer used for amino acid analysis and assayed in the amino acid analyser.

Fig. 1. Assay of galactosylhydroxy[^{14}C]lysine and glucosylgalactosylhydroxy[^{14}C]lysine by amino acid analyser

Cartilage cells (3×10^8) were incubated with $30 \mu\text{Ci}$ of [^{14}C]lysine in 14 ml of modified Krebs medium containing 20% foetal calf serum. After a 30 min incubation period the synthesis of collagen was stopped by adding cycloheximide and $\alpha\alpha'$ -bipyridyl and the sample was treated as described in the Experimental section and the Results section. After hydrolysis with 2M-NaOH, a part of the sample was hydrolysed in 0.20M-HCl for 3 h at 110°C and a part was hydrolysed as described in the Experimental section. (a) Standards of glucosylgalactosylhydroxylysine, galactosylhydroxylysine, hydroxylysine and lysine, assayed by ninhydrin reaction at 560 nm; (b) sample after NaOH hydrolysis; (c) sample after mild acid hydrolysis; (d) sample after strong acid hydrolysis.

Conditions for amino acid analysis

The amino acid analysis under standard conditions was carried out in a 63 cm column of a Jeol-5AH amino acid analyser at 64°C with 0.35M-sodium citrate buffer, pH 5.28 (Askenasi, 1973). The column was connected to a fraction collector and fractions (about 2 ml) were collected every 2 min. They were dissolved in 5 ml of Instagel (Packard Instrument Co., La Grange, IL, U.S.A.) and assayed in the liquid-scintillation counter. In some experiments the assay in the same column was started with 0.19M-sodium citrate buffer, pH 3.31, at 37°C. After 40 min the temperature was increased to 58°C and after 120 min the pH was changed to 4.23 (long programme). Fractions (about 5 ml) were collected for 5 min and they were dissolved in 10 ml of Instagel and assayed as above.

All ^{14}C -radioactivity counting was performed in a Wallac liquid-scintillation spectrometer with an efficiency of 80% and a background of 25 c.p.m.

Results

Assay of galactosylhydroxy[^{14}C]lysine and glucosylgalactosylhydroxy[^{14}C]lysine by amino acid analyser

Cartilage cells (3×10^8) were incubated for 30 min with 30 μCi of [^{14}C]lysine for 30 min, as described in

the Experimental section, and part of the sample was hydrolysed with NaOH and prepared for amino acid analysis. Other parts of the sample were hydrolysed further with either 0.20M- or 6M-HCl.

Several peaks of radioactivity were found in the amino acid analysis after hydrolysis with NaOH (Fig. 1b). One of them was eluted at 54 min, the position corresponding to that of glucosylgalactosylhydroxylysine standard (Fig. 1a). After mild acid hydrolysis this peak disappeared (Fig. 1c). One peak was eluted at 89 min after NaOH hydrolysis (Fig. 1b), like the galactosylhydroxylysine standard. After hydrolysis with 0.20M-HCl the size of this peak increased (Fig. 1c) and after hydrolysis with 6M-HCl this peak disappeared (Fig. 1d). Hydroxy[^{14}C]lysine was eluted at 165–175 min as a split peak (Fig. 1a and 1b). After mild or strong acid hydrolysis the size of this peak increased. [^{14}C]Lysine was eluted at 220–230 min (Figs. 1a and 1b).

In order to study whether the peaks of radioactivity that were eluted in the positions corresponding to glucosylgalactosylhydroxylysine and to galactosylhydroxylysine standards contained any non-specific radioactivity, one portion of the above sample after NaOH hydrolysis was analysed by using the long programme of the amino acid analyser, as described in the Experimental section. The ratio of radioactivity in the glucosylgalactosylhydroxylysine peak to that in the galactosylhydroxylysine peak was identical with the ratio obtained with the standard assay procedure (not shown).

Incorporation and hydroxylation of [^{14}C]proline by the cartilage cells

The synthesis and secretion of collagen by isolated cartilage cells were characterized by incubating the cells with [^{14}C]proline and by following the formation of hydroxy[^{14}C]proline in the cells and the appearance of non-diffusible hydroxy[^{14}C]proline in the medium. The incorporation of [^{14}C]proline was linear with time almost immediately after [^{14}C]proline was added to the incubation medium (not shown). The formation of hydroxy[^{14}C]proline was linear at about 4 min, and the secretion of collagen hydroxy[^{14}C]proline at about 35 min (Fig. 2).

Hydroxylation of [^{14}C]lysine and formation of galactosylhydroxy[^{14}C]lysine and glucosylgalactosylhydroxy[^{14}C]lysine by the cartilage cells

The incorporation of [^{14}C]lysine started almost immediately after [^{14}C]lysine was added to the incubation system (Fig. 3). The hydroxylation of [^{14}C]lysine was linear with time after about 4 min and hydroxy[^{14}C]lysine appeared in the medium after an incubation of about 35 min. The degree of hydroxylation of [^{14}C]lysine was about 7% in the cells and about

Fig. 2. Hydroxylation of [^{14}C]proline and secretion of collagen hydroxy[^{14}C]proline by the cartilage cells

Cartilage cells (8.0×10^7) were incubated in 22 ml of modified Krebs medium containing 20% foetal calf serum and 25 μCi of [^{14}C]proline. The non-diffusible hydroxy[^{14}C]proline was assayed in the cells and medium as described in the Experimental section. Values are the mean of duplicate samples from the same experiment. ○, Total non-diffusible hydroxy[^{14}C]proline in cells plus medium; ●, non-diffusible hydroxy[^{14}C]proline in the medium.

Fig. 3. Time-course for the incorporation of [^{14}C]lysine and hydroxylation of [^{14}C]lysine by the cartilage cells

Cartilage cells (1×10^8) were incubated in 14 ml of modified Krebs medium containing 20% foetal calf serum and $60 \mu\text{Ci}$ of [^{14}C]lysine. ○, Total non-diffusible ^{14}C -labelled protein in the cells plus medium; ●, total non-diffusible hydroxy[^{14}C]lysine in the cells plus medium; □, non-diffusible hydroxy[^{14}C]lysine in the medium.

Fig. 4. Glycosylation of hydroxy[^{14}C]lysine by cartilage cells

The conditions were the same as in Fig. 3. ●, Total hydroxy[^{14}C]lysine in the cells plus medium; ○, galactosylhydroxy[^{14}C]lysine in the cells plus medium given as the sum of galactosylhydroxy[^{14}C]lysine and glucosylgalactosylhydroxy[^{14}C]lysine; △, glucosylgalactosylhydroxy[^{14}C]lysine in the cells plus medium.

23% in the medium. The galactosylation of hydroxy[^{14}C]lysine and the glucosylation of galactosylhydroxy[^{14}C]lysine were linear at about the same time as the synthesis of hydroxy[^{14}C]lysine (Fig. 4),

Fig. 5. Incorporation and hydroxylation of [^{14}C]lysine when studied by a pulse-chase experiment in the cartilage cells

Cartilage cells (3.6×10^8) were incubated in 15 ml of modified Krebs medium containing 20% foetal calf serum. The cells were pulsed with $60 \mu\text{Ci}$ of [^{14}C]lysine for 5 min and the pulse was stopped by adding 1 ml of modified Krebs medium containing 3 mg of unlabelled lysine (indicated by the arrow). At each time-point, 2 ml of the incubation system (cells plus medium) was taken and analysed for total non-diffusible radioactivity and for substituted and non-substituted hydroxy[^{14}C]lysine as described in the Experimental section. ○, Total non-diffusible radioactivity in the cells plus medium; ●, total non-diffusible hydroxy[^{14}C]lysine in the cells plus medium.

and no definite time-lag could be observed between the hydroxylation and the glycosylations.

The experiments were continued by carrying out pulse-chase experiments. Cartilage cells (3.6×10^8) were pulsed with $60 \mu\text{Ci}$ of [^{14}C]lysine for 5 min and the pulse was stopped by adding 3 mg of unlabelled lysine. Small samples were taken after the incubation times indicated, and at each time-point total non-diffusible radioactivity and hydroxy[^{14}C]lysine and its glycosides were assayed. The total radioactivity did not increase significantly during the chase period (Fig. 5). The hydroxylation of [^{14}C]lysine increased during the chase period for about 20 min and was relatively constant thereafter. The galactosylation and glucosylation of hydroxy[^{14}C]lysine increased likewise for about 20 min during the chase period and remained constant thereafter (Fig. 6a). The increase in the amount of glucosylgalactosylhydroxy[^{14}C]lysine was larger than the increase in the amounts of galactosylhydroxy[^{14}C]lysine and non-substituted hydroxy[^{14}C]lysine during the chase period (Fig. 6b). The ratio of substituted hydroxy[^{14}C]lysine to total hydroxy[^{14}C]lysine was 0.77 in the cells after a 30 min chase period, and 0.65 in the medium after a 60 min chase period.

Fig. 6. Glycosylation of hydroxy[¹⁴C]lysine when studied by a pulse-chase experiment in the cartilage cells

The experiment is the same as in Fig. 5. (a) ●, Total non-diffusible hydroxy[¹⁴C]lysine in cells plus medium; ○, total non-diffusible galactosylhydroxy[¹⁴C]lysine in the cells plus medium given as the sum of galactosylhydroxy[¹⁴C]lysine and glucosylgalactosylhydroxy[¹⁴C]lysine; △, total non-diffusible glucosylgalactosylhydroxy[¹⁴C]lysine in the cells plus medium. (b) ●, Unsubstituted hydroxy[¹⁴C]lysine in the cells plus medium; ○, galactosylhydroxy[¹⁴C]lysine in the cells plus medium; △, glucosylgalactosylhydroxy[¹⁴C]lysine in the cells plus medium. In both (a) and (b) the addition of unlabelled lysine is indicated by an arrow.

Discussion

Previous studies on isolated cartilage cells have indicated that in continuous labelling experiments the incorporation of proline becomes linear after about 2 min, the hydroxylation of proline after about 4 min, and the secretion of collagen after about 37 min (Dehm & Prockop, 1973). Pulse-chase experiments with similar cells have further indicated that after a 4 min pulse-label a chase period of more than 12 min or about 18 min is required for maximal disulphide bonding and that the triple-helix formation occurs

soon thereafter (Uitto & Prockop, 1974; Harwood *et al.*, 1975a). In the continuous labelling experiments of the present study the hydroxylation of [¹⁴C]proline became linear after about 4 min and the secretion of collagen hydroxy[¹⁴C]proline after about 35 min, suggesting that the time-course for events in the intracellular collagen biosynthesis was similar to that reported previously (Dehm & Prockop, 1973).

In the assay of the labelled compounds by amino acid analyser several peaks of radioactivity were found. However, all compounds studied here could be separated from the extra peaks and experiments with different hydrolysis conditions confirmed the identification of the hydroxylysine glycoside peaks. A similar presence of extra peaks of radioactivity after labelling with [¹⁴C]lysine had been previously reported by Brownell & Veis (1975). Because hydroxylysine and the hydroxylysine glycosides are found only in collagen, no purification of the protein itself was required. However, lysine is present in many other proteins too and thus the ratio of hydroxy[¹⁴C]lysine to total radioactivity was lower than that reported for purified collagen.

In the continuous labelling experiments with [¹⁴C]lysine, the formation of hydroxy[¹⁴C]lysine was linear after about 4 min and thus the hydroxylation of lysine was linear at about the same time as the hydroxylation of proline. The galactosylation of hydroxylysine and the glucosylation of galactosylhydroxylysine were linear after about the same time. Previous studies have indicated that the hydroxylations of proline and lysine residues are initiated while the polypeptide chains are growing on the ribosomes (see the introduction). Because no significant lag could be found between the hydroxylation of lysine and the glycosylation of hydroxylysine, the present data suggest that the glycosylations are also initiated while the polypeptide chains are growing on the ribosomes. This suggestion is in agreement with recent data on the intracellular location of these glycosylation reactions in cultured chick-embryo fibroblasts (Brownell & Veis, 1975). The data are also consistent with previous findings that indicated that there is apparently no significant lag period between the hydroxylation of lysine residues and the glycosylation of hydroxylysine residues during the biosynthesis of basement-membrane collagen (Grant *et al.*, 1972; Clark *et al.*, 1975).

The pulse-chase experiments provided further information on the time-courses of the hydroxylation of lysine residues and the glycosylations of hydroxylysine residues. After a 5 min pulse-label, the hydroxylation and the two glycosylations continued during the chase period for up to about 20 min, but no further increase in the formation of the products of any of these reactions occurred after this time-point. This is the time-point when the triple helix is formed during collagen biosynthesis in the cartilage cells, and the

molecules then pass from the cisternae of the endoplasmic reticulum into the Golgi vacuoles (see the introduction). The synthesis time of a pro- α chain in the cartilage cell is about 6min (Miller *et al.*, 1973). Since it seems unlikely that the chains would remain bound to the ribosomes until the 20min time-point during the chase period, the present data suggest that the hydroxylation of lysine residues and the glycosylations of hydroxylysine residues in the cartilage cells are continued within the cisternae of the endoplasmic reticulum after the release of completed polypeptide chains from the ribosomes. Because none of these reactions were continued after about 20min, it seems probable that no significant hydroxylation of lysine residues or glycosylation of hydroxylysine residues took place within the Golgi vacuoles. The data are consistent with reports on the properties of lysine hydroxylase (Kivirikko *et al.*, 1973; Ryhnen & Kivirikko, 1974), collagen galactosyltransferase (Risteli *et al.*, 1976) and collagen glucosyltransferase (Myllyl *et al.*, 1975b), indicating that these enzymes do not catalyse the reactions with native collagen consisting of triple-helical molecules. During the preparation of the present paper, Harwood *et al.* (1975c) reported data in different experiments with cartilage cells, which likewise suggest that the collagen-glycosylation reactions are initiated on growing peptide chains attached to membrane-bound ribosomes, and that these reactions are essentially complete when the molecules have moved into the smooth endoplasmic reticulum and Golgi vacuoles.

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References

- Anttinen, H. & Kivirikko, K. I. (1976) *Biochim. Biophys. Acta* in the press
- Askenasi, R. (1973) *Biochim. Biophys. Acta* **304**, 375–383
- Bornstein, P. (1974) *Annu. Rev. Biochem.* **43**, 567–603
- Brownell, A. G. & Veis, A. (1975) *Biochem. Biophys. Res. Commun.* **63**, 371–377
- Clark, C. C., Tomich, E. A., Koszalka, T. R., Minor, R. R. & Kefalides, N. A. (1975) *J. Biol. Chem.* **250**, 5259–5267
- Dehm, P. & Prockop, D. J. (1971) *Biochim. Biophys. Acta* **240**, 358–369
- Dehm, P. & Prockop, D. J. (1973) *Eur. J. Biochem.* **35**, 159–166
- Grant, M. E., Kefalides, N. A. & Prockop, D. J. (1972) *J. Biol. Chem.* **247**, 3539–3544
- Harwood, R., Grant, M. E. & Jackson, D. S. (1973) *Biochem. Biophys. Res. Commun.* **55**, 1188–1196
- Harwood, R., Bhalla, A. K., Grant, M. E. & Jackson, D. S. (1975a) *Biochem. J.* **148**, 129–138
- Harwood, R., Grant, M. E. & Jackson, D. S. (1975b) *Biochem. Soc. Trans.* **3**, 136–137
- Harwood, R., Grant, M. E. & Jackson, D. S. (1975c) *Biochem. J.* **152**, 291–302
- Juva, K. & Prockop, D. J. (1966) *Anal. Biochem.* **15**, 77–83
- Katzman, R. L., Halford, M. H., Reinhold, V. N. & Jeanloz, R. W. (1972) *Biochemistry* **11**, 1161–1167
- Kivirikko, K. I., Ryhnen, L., Anttinen, H., Bornstein, P. & Prockop, D. J. (1973) *Biochemistry* **12**, 4966–4971
- Martin, G. R., Byers, P. H. & Piez, K. A. (1975) *Adv. Enzymol.* **42**, 167–191
- Miller, E. J. (1971) *Biochemistry* **10**, 1652–1659
- Miller, E. J., Woodall, D. L. & Vail, M. S. (1973) *J. Biol. Chem.* **248**, 1666–1671
- Myllyl, R., Risteli, L. & Kivirikko, K. I. (1975a) *Eur. J. Biochem.* **52**, 401–410
- Myllyl, R., Risteli, L. & Kivirikko, K. I. (1975b) *Eur. J. Biochem.* **58**, 517–521
- Olsen, B. R., Berg, R. A., Kishida, Y. & Prockop, D. J. (1975) *J. Cell Biol.* **64**, 340–355
- Prockop, D. J., Berg, R. A., Kivirikko, K. I. & Uitto, J. (1976) in *Biochemistry of Collagen* (Ramachandran, G. N. & Reddi, A. H., eds.), Plenum Publishing Corp., New York, in the press
- Risteli, L., Myllyl, R. & Kivirikko, K. I. (1976) *Biochem. J.* **155**, 145–153
- Ryhnen, L. & Kivirikko, K. I. (1974) *Biochim. Biophys. Acta* **343**, 129–137
- Schofield, J. D. & Prockop, D. J. (1973) *Clin. Orthop. Relat. Res.* **97**, 175–195
- Schofield, J. D., Uitto, J. & Prockop, D. J. (1974) *Biochemistry* **13**, 1801–1806
- Uitto, J. & Prockop, D. J. (1973) *Biochem. Biophys. Res. Commun.* **55**, 904–911
- Uitto, J. & Prockop, D. J. (1974) *Biochemistry* **13**, 4586–4591