Amino-acid Sequence of a High-sulphur Protein from Wool

THE high-sulphur proteins of α-keratins, which constitute the non-filamentous matrix between the microfibrils, comprise several major groups of proteins, each group consisting of a number of closely related components. They are obtained in a soluble form by reduction of the disulphide bonds of wool and preferential extraction with alkaline thioglycollate at high ionic strength[1]. The thiol groups are subsequently stabilized by alkylation with iodoacetic acid.

Three members of one such group (components *A*, *B* and *C*) have been isolated from the high-sulphur fraction of Lincoln wool (my unpublished work with H. Lindley). All are rich in S-carboxymethylcysteine (~25%), lack lysine, histidine and methionine, and have common structural features. This paper reports the amino-acid sequence of component *C* shown in Fig. 1.

The most significant feature of the sequence is the triplication of a ten residue sequence between residues 21 and 52 and further repetitions of a similar unit in the latter half of the molecule. These homologies (Fig. 1) suggest that the gene for this molecule was derived from an ancestral gene specifying a much smaller protein. The mechanism by which this ancestral gene evolved would have included several partial gene duplications and triplications[2].

It is of considerable interest that a repeating unit has been found in a protein from the high-sulphur fraction of wool, which comprises the interfibrillar matrix (a structure often considered to be composed of disorganized polypeptide chains) because repeating units in other fibrous proteins are associated with highly structured features.

The silk fibroin of *Bombyx mori*, the silkworm, is composed of antiparallel chain pleated sheets, formed from polypeptide chains in which the amino-acid residues are alternately glycine and alanine (or serine)[3]. The collagen helix, likewise, is dependent on the repeating unit Gly-Pro-X[4]. The supercoiling of the α-helices in the k-m-e-f class of proteins[5], and the helical array of antiparallel chain pleated sheet subunits in feather keratin[6], imply considerable sequence restrictions and the presence of repetitive interacting units in these structures.

The repetitive sequences in this high-sulphur protein, by analogy with the repeating structures of other fibrous proteins, indicate that structured features in the matrix of wool are quite probable.

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[1] Gillespie, J. M., *Austral. J. Biol. Sci.*, **15**, 262 (1962).
[2] Dixon, G. H., in *Essays in Biochemistry* (edit. by Campbell, P. N., and Greville, G. D.), **2**, 147 (Academic Press, London, 1966).
[3] Zahn, H., Schade, W., and Ziegler, K., *Biochem. J.*, **104**, 1019 (1967).
[4] Schroeder, W. A., Kay, L. M., Legette, J., Honnen, L., and Green, F. C., *J. Amer. Chem. Soc.*, **76**, 3556 (1954).
[5] Cohen, C., and Holmes, K. C., *J. Mol. Biol.*, **6**, 423 (1963).
[6] Fraser, R. D. B., MacRae, T. P., Parry, D. A. D., and Suzuki, E., *Polymer*, **12**, 35 (1970).

Synchrony of Long Duration in Suspension Cultures of Mammalian Cells

SYNCHRONOUS growth of mammalian cell cultures may be induced either by reversible inhibition of DNA synthesis[1,2] or by physically separating cells in one specific phase of the division cycle[3–9] and incubating them separately. In mammalian cell cultures synchrony is usually subject to relatively rapid decay and, in general, synchronous proliferation is studied for not longer than two cell generations. Observations of longer duration revealed rapid dampening of rhythmic oscillations in cell division rate[4,10]. Cultures maintaining synchronous growth during prolonged periods of time would, however, be useful for further characterization of various kinetic aspects of proliferative behaviour, in particular of the variation of generation times within a cell population.

Here we describe a cell culture system in which synchrony is sufficiently well maintained to be detectable during 8–12 consecutive cell cycles. Cultures of a transplantable murine mast cell tumour (cell line P-815-X2) were used. The origin of the cell line and the method of preparing synchronously multiplying cell populations have been described in detail[6]. In brief, cells in early interphase were separated from the rest of the population by sucrose density gradient centrifugation and reincubated in steady state conditions. The distribution within

1	Acetyl Ala	Cmc	Cmc	Ser	Thr	Ser	Phe	Cmc	Gly	Phe
11	Pro	Ile	Cmc	Ser	Thr	Ala	Gly	Thr	Cmc	Gly
21	Ser	Ser	Cmc	Cmc	Arg	Ser	Thr	Cmc	Ser	Gln
31	Thr	Ser	Cmc	Cmc	Gln	Pro	Thr	Ser	Ile	Gln
41	Thr	Ser	Cmc	Cmc	Gln	Pro	Thr	Cmc	Leu	Gln
51	Thr	Ser	Gly	Cmc	Glu	Thr	Gly	Cmc	Gly	Ile
61	Gly	Gly	Ser	Ile	Gly	Tyr	Gly	Gln	Val	Gly
71	Ser	Ser	Gly	Ala	Val	Ser	Ser	Arg	Thr	Arg
81	Trp	Cmc	Arg	Pro	Asp	Cmc	Arg	Val	Glu	Gly
91	Thr	Ser	Leu							
94	Pro	Pro	Cmc	Cmc	Val	Val	Ser	Cmc	Thr	Ser
104	Pro	Ser	Cmc	Cmc	Gln	Leu	Tyr	Tyr	Ala	Gln
114	Ala	Ser	Cmc	Cmc	Arg	Pro	Ser	Tyr	Cmc	Gly
124	Gln	Ser	Cmc	Cmc	Arg	Pro	Ala	Cmc		
132			Cmc	Cmc	Gln	Pro	Thr	Cmc	Thr	Glu
140		Pro	Val	Cmc	Glu	Pro	Thr	Cmc	Ser	Gln
149	Pro	Ile	Cmc							

Fig. 1 The complete amino-acid sequence of component *C* of the high-sulphur fraction of Lincoln wool. Homologous residues are underlined, and the sequence has been arranged to show maximum homology.