The Vertebrate Eye Lens

A useful system for the study of fundamental biological processes on a molecular level.

Hans Bloemendal

The vertebrate eye lens represents an unusual but efficient tissue for the elucidation of a number of representative biological processes. In this article attention is directed to the highly specific, polymeric protein, α-crystallin, whose fate, after synthesis, can be followed by post-translational modifications and self-assembly. This protein, a major constituent of all mammalian species, can easily be purified and is very suitable for comparative studies of amino acid sequences.

Lens cells are one of the best sources for the purification of plasma membranes and their junctions. The availability and characterization of these membranes should assume ever greater importance as more information is acquired on cellular transport.

In that lens proteins and nucleic acids derived from calf eye have been studied intensively (*1*), the major part of the results reviewed in this article are taken from studies on calf lens tissue.

The vertebrate eye lens is a highly specialized organ whose sole function is to carry out proper refraction of incident light beams in order to ensure visual acuity. The organ, which is completely devoid of blood vessels, gets its nourishment from the surrounding fluid, called the aqueous humor.

The lens is generated from ectodermal cells, and is composed of this cell type in its various differentiated forms. The outer epithelial monolayer contains the only cell population that has mitotic activity. In the epithelium, there is a region of cellular elongation where initiation of the differentiation into so-called fiber cells occurs (*2*). Shortly after elongation begins, nuclei and mitochondria disappear from the lens fibers. The disappearance of nuclei has been studied extensively (*3*). During this process DNA in the condensed chromatin is broken into smaller pieces. The disappearance of mitochondria, although not yet fully clarified, may, as judged by electron micographs of Jurand and Yamada (*4*), be due to mitochondrial extrusion. However, the peripheral layer of the lens fibers still contains the complete protein-synthesizing machinery.

The lens grows throughout the whole life span of the animal. Its rate of growth is rapid in embryonic development and early postnatal life, but gradually slows down as the growth of the whole organism slows down. Only very few mitoses are seen in epithelial cells of the adult, but these cells do divide at a rather high rate when the lens is injured.

The cells that differentiate from the epithelium are laid down in layers at the periphery (or outer cortex) of the organ and are then displaced toward the center, eventually resulting in a structure in which distinct layers can be recognized. These layers are formed at different stages of life and, accordingly, are called the embryonic, fetal and adult-nuclear, and cortical layers (*5*) (Fig. 1). The core (or nucleus) still contains the cells of the embryonic stage since lens cells are preserved as long as the animal is alive. The continuous process of cell division in the lenticular epithelium leads not only to a progressive size of the lens, but also to a gradual hardening of the nucleus while the organ ages. Human lenses, however, maintain a water content of about 65 percent throughout life.

In view of its special features, it is quite conceivable that the lens is extremely useful for studies, on a molecular level, of fundamental processes such as growth, differentiation, and aging. In all these processes one of the major lens proteins, α-crystallin, can be used as a marker. For example, with differentiation and increasing age, changes occur in subunit composition, in physical characteristics, and in packing density. For these reasons and because α-crystallin has, of all lens proteins, most intensively been studied, I shall restrict the discussion mainly to this protein. However, brief mention has to be made first of the general protein composition in which α-crystallin plays its major role.

Protein Composition

The chemical composition of the eye lens is unusual in that about 35 percent of its wet weight is protein, whereas the remaining 65 percent is virtually water. Water-soluble lens proteins—which are called crystallins—contribute more than 90 percent of the total lens protein. They can easily be separated by gel filtration on Sephadex G-200, Ultrogel AcA 34, or Biogel P300 columns into four major classes of crystallin: α, β_H, β_L, and γ (Fig. 2). This high amount of a rather limited number of very specialized proteins that are made both in the epithelium and in the cortical fiberlike cells facilitates the analysis of the biosynthetic events. The lens is even more attractive for the study of protein synthesis, since organ culture experiments revealed that about 50 percent of the total synthesis is directed toward the production of α-crystallin (*6*).

Interest in the characterization of the water-insoluble components of the lens has recently arisen because, in addition to insoluble protein described earlier (*7*), this fraction contains lens plasma membranes (*8, 9*). The formation of lens fiber plasma membranes is an unresolved problem and is important because of the significance of membrane changes in cellular differentiation.

Characterization of the water-insoluble lens proteins has only been started recently (*10, 11*). Some of the membrane proteins can be dissolved if the water-insoluble part of the lens is treated with 7*M* urea. However, a consistent amount of protein still remains associated to membranous structures that can be solubilized by treatment with sodium dodecyl sulfate (SDS). The corresponding patterns obtained after SDS–polyacrylamide gel electrophoresis, which, together with the profile of the water-soluble crystallins, represent the total lens protein population, are shown in Fig. 3. These differential solubilization properties and the complex feature of the electrophoretic profiles of the various fractions suggest a multiple association between protein and other constituents of the lens.

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α-Crystallin

The development and aging of the lens, the only organ that never sheds cells, is accompanied by a gradual change in subunit composition of its structural proteins. The most intensively studied and best characterized lens protein is calf α-crystallin, which has long been considered a well-defined homogeneous protein. In recent years, however, new data became available which demonstrated unequivocally that, what was thought to be a single high-molecular-weight protein, is, in fact, a whole population of aggregates of varying size, albeit with an average molecular weight of about 800,000 (*12*). Aged α-crystallin is sometimes called HM-α-crystallin (HM stands for high molecular weight). It is formed by aggregates with a molecular weight of many millions (*13*). A possible role in the formation of high-molecular-weight α-crystallin aggregates has been ascribed to such different substances as glucose (*14*) or calcium (*15*). But the true cause of the age-dependent aggregation is still unknown. Despite the fact that the native α-crystallin is not a protein of uniform molecular size, each individual aggregate isolated from adult lens fibers consists basically of the same four kinds of polypeptide chains, designated αA_1, αA_2, αB_1, and αB_2 (*16*) (Fig. 4) [A stands for acidic, B for basic (*17*)]. Upon aging, a breakdown takes place starting from the COOH-terminus (Fig. 13). Quantitatively the αA_2 polypeptide is the most predominant subunit. The individual chains can be isolated by column chromatography (*18*) or preparative isoelectric focusing (*19*) after dissociation of α-crystallin in 7*M* urea. The electrophoretic pattern of the α-crystallin chains in 7*M* urea containing polyacrylamide gels is shown in Fig. 4. The four different subunits are completely separated, and the intensity of staining of the individual bands reflects their relative amounts. However, in gels that contain SDS the αA_1 chains coincide with αA_2. Also, the two B chains cannot be separated in this medium. While urea gel electrophoresis provides the correct information that the net charges of each of the four chains differ, the SDS gel pattern suggests equal molecular weights for the A chains and somewhat higher, but again identical, molecular weights for the two B chains (Fig. 5). In contrast to this result, the complete amino acid sequence (see, for example, Fig. 7) leads to the conclusion that both types of chains have about the same molecular weight; αA_2 with 173 amino acid residues has a molecular weight of 19,832 (*20*), and αB_2 with 175 amino acid residues has a molecular weight of 20,070 (*21*). It is necessary, therefore, to assume that some conformational difference may exist between A and B chains, and that SDS does not lead to complete unfolding of the αB polypeptides. This assumption is supported by the observation that the difference in mobilities between A and B chains virtually disappears in SDS gels containing 7*M* urea (*22*).

As was stated before, α-crystallin results from the aggregation of four major monomeric subunits, of which only two (αA_2 and αB_2) are under direct genetic control (*23*). Hence the formation of α-crystallin can serve as a model for the study of the biosynthesis of a polymeric protein of extremely high molecular weight.

The α-Crystallin Messengers

The information for determining the amino acid sequence in a polypeptide is stored in the sequence of nucleotides of DNA in the genome. Synthesis of messenger RNA (mRNA) is achieved by transcription of the DNA sequence into RNA. This synthesis can be inhibited by the antibiotic actinomycin D. When embryonic chick lenses were treated with this drug, however, messenger activity was preserved in the lens cortex (*24*), suggesting the presence of mRNA with extremely high stability. Similar results were obtained with calf lens (*25*). This may be due either to the lack of ribonuclease or to the presence of a relatively high concentration of a potent ribonuclease inhibitor. Indeed, the lens cortex is an extremely good source for the isolation of a ribonuclease inhibitor (*26*), a protein whose properties are similar to that described in rat liver (*27*). Furthermore, low ribonuclease concentration has been demonstrated in lens tissue (*28*). Since approximately 3 percent of α-crystallin in the outer lens fibers is renewed daily in the absence of RNA synthesis, it may again be concluded that a stable messenger population operates in this part of the lens. It has been shown in experiments in vitro that in the cortex 75 percent of the total protein biosynthesis is due to the formation of α-crystallin (*29*). From this observation, it was obvious that a viable α-crystallin messenger should be obtainable from the cortical lens fibers. Actually the messenger for the αA_2 chain was one of the first eukaryotic mRNA species that could be shown to direct the synthesis of a single specific polypeptide in vitro (*30*, *31*) and in vivo (*32*).

The isolation of calf lens polyribosomes active in amino acid incorporation was reported 10 years ago (*33*). Evaluation of the electron micrographs taken from a great number of different preparations revealed an average size of six ribosomes per polysome (*34*), in accordance with the size of crystallin polypeptides that are made on those polysomes (the molecular weight ranging from 20,000 to 30,000). These polysomes are the starting material for the isolation of mRNA for crystallin. After dissociation of the polysomes by treatment with SDS, a single purification step in a zonal centrifuge yields two size classes of lens protein mRNA (*35*) (Fig. 6). Additional purification was achieved by recentrifugation, by affinity chromatography on oligodeoxythymidylate [oligo(dT)]- or poly(dT)-cellulose, and eventually by polyacrylamide gel electrophoresis in formamide (*36*). The two mRNA fractions obtained after zonal centrifugation through an isokinetic sucrose gradient are characterized by the sedimentation coefficients of 10*S* and 14*S*, respectively. Especially, the 14*S* mRNA is of interest,

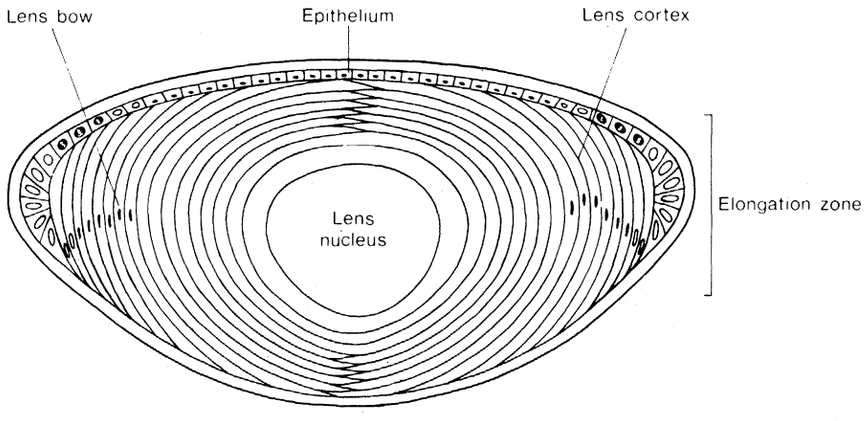


Fig. 1. Schematic drawing of a mammalian eye lens. Mitotic activity is restricted to the epithelial cells in the outer monolayer. The long fiberlike cells are arranged in an onionlike structure around the nucleus, which contains the fibers of the embryonic stage.

since assays of the isolated mRNA in various heterologous cell-free systems (*30, 37*) or in living oocytes (*32*) revealed that only the synthesis of the major subunit αA_2 is directed by the 14*S* mRNA. In contrast, the αB_2 mRNA is found in the 10*S* fraction, which also directs the synthesis of several crystallin chains that move, in coelectrophoresis, with β and γ chains (*31*). This group of lens mRNA's has not yet been fractionated to purity.

The 14*S* and 10*S* mRNA fractions can also be isolated from discrete ribonucleoprotein (RNP) particles with sedimentation values of about 21*S* and 16*S*, respectively (*38*). Those mRNP particles were obtained after dissociation of the polysomes with EDTA followed by gradient centrifugation. Puromycin-induced lens mRNP's have been reported to have somewhat lower sedimentation coefficients, namely 19*S* and 13*S*, respectively (*39*).

The high sedimentation value of the 14*S* crystallin mRNA is still a mystery. Although it is common to eukaryotic mRNA's to have, in addition to the nucleotide sequence that encodes the amino acid sequence of the polypeptides, so-called noncoding regions, the number of nucleotides available for these regions in αA_2 mRNA is extraordinarily high. One arrives at this conclusion by a simple calculation. αA_2-Crystallin contains 173 amino acid residues (*20*). Like other eukaryotic mRNA's, the 14*S* lens mRNA has a polyadenylic acid [poly(A)] track at the 3′ end with an approximate length of 150 nucleotides (*40*). Since the molecular weight of the 14*S* mRNA is about 500,000, corresponding to approximately 1,400 nucleotides, there are more than 700 nucleotides left with an unknown function. As long as experimental proof is lacking, one can only speculate about the meaning of the excess nucleotides in the αA_2-crystallin mRNA. There are several interesting possibilities that would explain the large size of the 14*S* mRNA:

1) It might be bicistronic, that is, it could code for two identical or two different polypeptide chains. However, until now no eukaryotic bi- or polycistronic mRNA's are known with more than one available initiation site. It is possible, although not experimentally verified, that the 10*S* crystallin mRNA is part of the 14*S* mRNA and is generated by nucleotide cleavage. This theory is attractive, since the αA and αB chains have a high degree of homology (*21*). The gene for the A chain might have been duplicated early in evolution and transcribed as one mRNA species coding for A chains. Only after nucleolytic splitting the mRNA for the B chains becomes operative. A similar processing has recently been observed for various viral mRNA's (*41*).

2) The 14*S* mRNA codes for a larger precursor protein whose size is reduced in a posttranslational process. Similar precursors have been described for other mRNA's (*42*), although there the excess nucleotides are significantly less. In case of lens polypeptides there is no evidence that a large precursor of αA_2 does exist, although processing might occur so fast that it escapes observation during short-time labeling experiments (pulse-chase).

3) It might well be that most of the calculated excess nucleotides are involved in a regulatory structure or represent just an "accident of nature." The latter possibility is rather unlikely, since in different species—for example, chicken (*43*), calf (*36*), and rat (*44*)—the mRNA for αA_2 polypeptide chains is relatively long. As has been mentioned, it is quite puzzling that the αB_2 chain, consisting of 175 amino acid residues and showing an almost 60 percent homology with the αA_2 polypeptide (*21*) (Fig. 7), is encoded by a 10*S* mRNA. Here the ratio of the length of the mRNA to the length of the encoded polypeptide is in accord with the findings for globin mRNA (*45*).

Regions of secondary structure have been observed in various mRNA's (*46*), including the 14*S* lens mRNA (*40*). If the excess nucleotides really represent secondary structures that are not involved in coding, they have been extremely conservative in evolution, at least as far as their dimension is concerned. This hy-

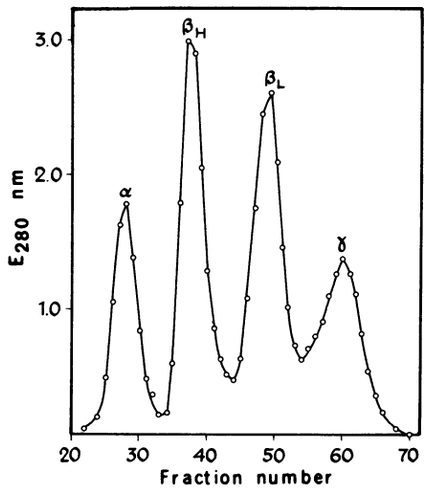


Fig. 2. Fractionation of calf lens crystallins by gel filtration on an Ultrogel AcA 34 column. α-Crystallin emerges with the void volume. β-Crystallin is separated into a class of high-molecular-weight (β_H) and lower-molecular-weight (β_L) protein, respectively. γ-Crystallin, a mixture of related monomeric proteins contains a β-crystallin species called β_S. (As compared to gel filtration on a Sephadex G200 column the time of separation can be reduced by a factor of 2, with Ultrogel AcA 34 as packing material of the column.)

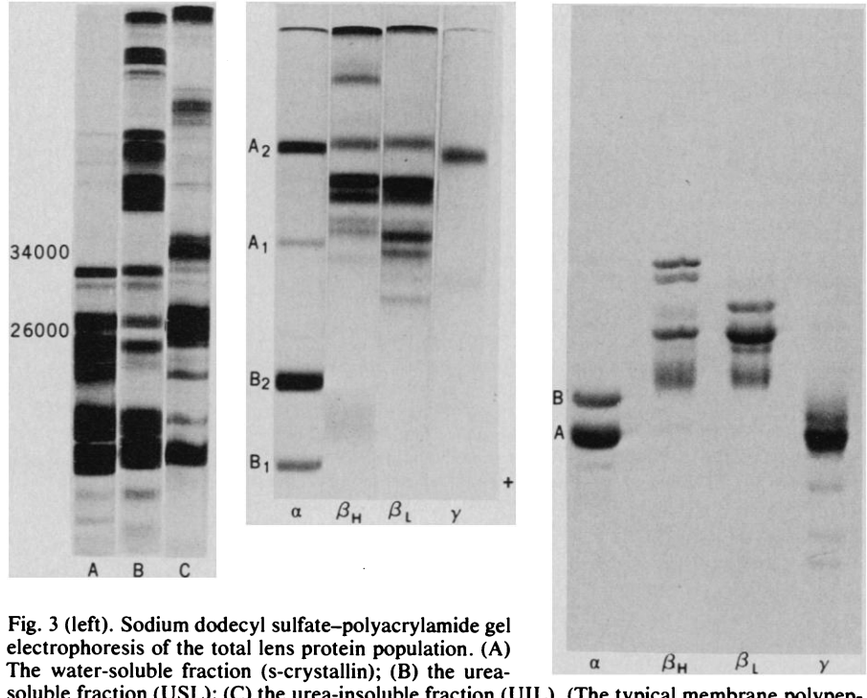


Fig. 3 (left). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the total lens protein population. (A) The water-soluble fraction (s-crystallin); (B) the urea-soluble fraction (USL); (C) the urea-insoluble fraction (UIL). (The typical membrane polypeptides are in the molecular-weight regions of 26,000 and 33,000 to 34,000). Fig. 4 (center). Urea-polyacrylamide gel electrophoresis pattern of the polypeptide chains of α-crystallin. For comparison, the chain distribution pattern of the other crystallins is also shown. Fig. 5 (right). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the separated crystallins.

pothesis can be derived from our investigations with rat lens crystallin (*44*). The αA_2 chain of calf and rat lens are closely related because these polypeptides differ only in six amino acids (*47*). This means that the coding regions of the rat lens αA_2 mRNA are almost identical to those of the calf lens αA_2 mRNA. Whether or not the noncoding regions are similar cannot be stated at present, but the observation has been made that the rat αA_2 chain is also coded for by an 14*S* mRNA. Rat lens α-crystallin appeared to have, in addition to the αA and αB subunits, a polypeptide that is identical in its NH_2- and COOH-terminal sequences to αA_2 but differs in that it has an insertion of about 20 amino acid residues. For the time being, this polypeptide, with a molecular weight of approximately 24,000 (*44*) (Fig. 8), has been designated as αX. Also, this polypeptide seems to be coded for by an mRNA species sedimenting on sucrose gradients in the 14*S* region.

α-Crystallin is not only useful for the study of the translation of eukaryotic mRNA's as an overall process. It can also serve as a model for the investigation of details of the process, such as the initiation step and the phenomenon of NH_2-terminal acetylation.

Initiation of α-Crystallin Chains

The elucidation of the complete primary structure of the A and B chains of α-crystallin confirmed the previous finding that the NH_2-terminal peptide of all α-crystallin subunits is *N*-acetyl-Met-Asp-Ile-Ala (*48, 49*) (Fig. 7). Protein biosynthesis in prokaryotes as well as in eukaryotes is started with the aid of initiator methionyl transfer RNA ($tRNA^{Met}$) (formylated in prokaryotes). Therefore, it was interesting to know whether the NH_2-terminal methionine of α-crystallin is derived from this initiator transfer RNA (tRNA), or, alternatively, that an original sequence Met-Met-Asp-Ile-Ala--- was trimmed by an aminopeptidase to yield Met-Asp-Ile-Ala followed by NH_2-terminal acetylation of the penultimate methionine, which was donated by $Met\text{-}tRNA^{Met}$. Proposals suggesting that protein synthesis in eukaryotes is initiated by acetylated amino acids (*50*) or as a special case for histones (*51*) have been refuted (*52*). Nevertheless, this abandoned idea has recently been put forward again (*53*). Experiments in our laboratory demonstrated unequivocally that the NH_2-terminal methionine of α-crystallin chains is donated exclusively by $tRNA^{Met}_f$ which is responsible for initiation and not by $tRNA^{Met}$, which provides methionine residues for internal positions in a polypeptide chain (*54*). Moreover, we were able to show that acetyl-$Met\text{-}tRNA^{Met}_f$ cannot replace formyl-$Met\text{-}tRNA^{Met}_f$ (or the unformylated species) in the initia-

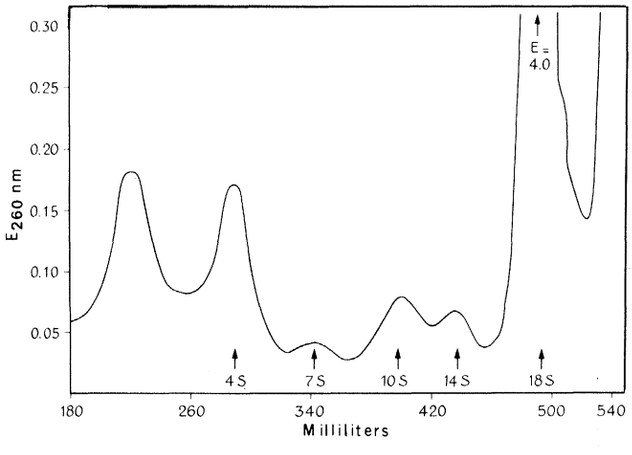
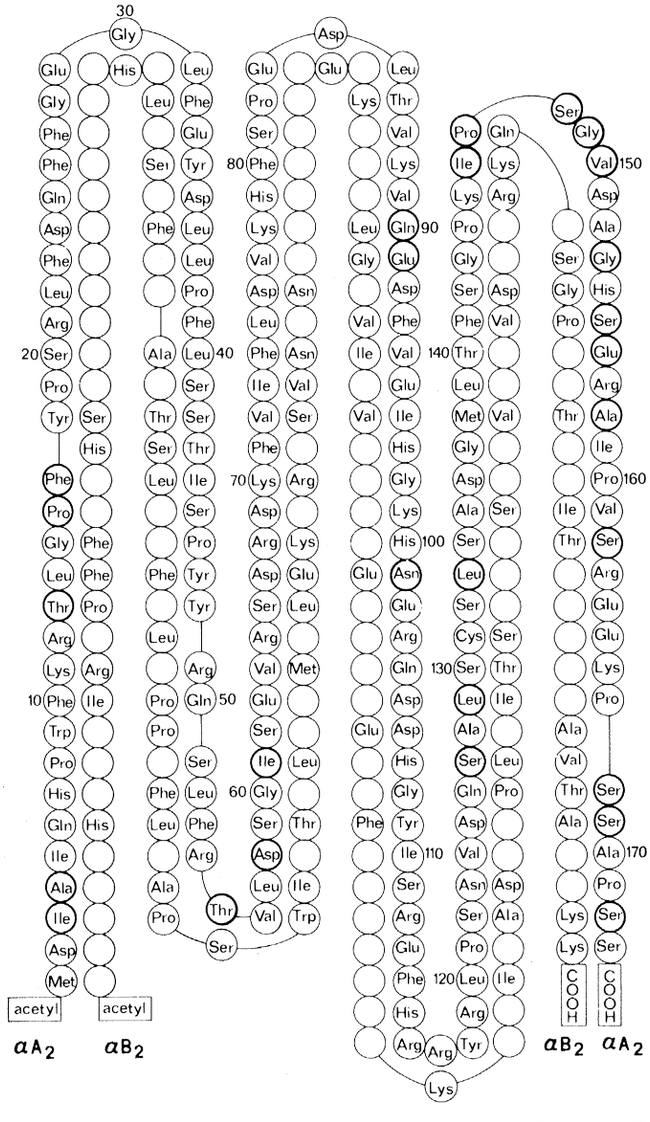


Fig. 6 (above). Pattern obtained after zonal centrifugation of polysomal RNA from calf lens. The crystallin messenger fractions are located in the 10*S* and 14*S* region. Fig. 7 (right). The primary structure of the α-crystallin polypeptide chains. There is a high degree of homology between the A and B chains (identical amino acid residues in the B chain are visualized by empty circles). The boldface circles represent amino acid residues that are different in the A_2 chains of man, rhesus monkey, rabbit, rat, cat, dog, rhinoceros, horse, pig, and kangaroo (*47*).



tion of lens crystallins (Fig. 9). This latter observation was the first indication that NH_2-terminal acetylation of α-crystallin is a postinitiation process.

NH_2-Terminal Acetylation of α-Crystallin

After it was demonstrated that the NH_2-terminal methionine of crystallin chains was the residue donated by the initiator tRNA, the question remained whether acetylation took place immediately after initiation, during chain elongation, or after release of the completed polypeptide. In order to solve this problem lens polysomes were isolated after a short incubation of a lens cell-free system with [^{35}S]Met-tRNA$_f^{Met}$ followed by cycloheximide treatment, which prevents further polypeptide synthesis. The growing chains were dissociated from the ribosomes and fractionated according to size by gel chromatography (*54*). It appeared that only those peptides synthesized de novo and having a minimal length corresponding to about 25 amino acid residues carried an acetyl group in NH_2-terminal position. These observations provided strong evidence that acetylation occurs on growing polypeptide chains after they are sufficiently long to protrude from the ribosome. The NH_2-terminal acetylation of proteins takes

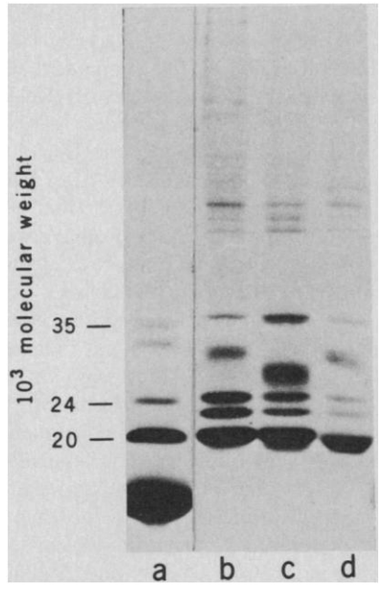
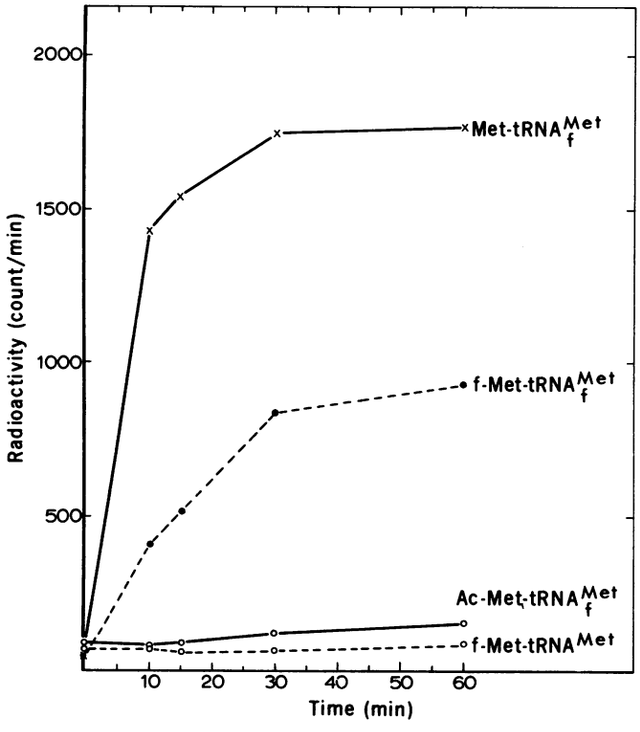


Fig. 8. Autoradiography of sodium dodecyl sulfate gel electrophoretic patterns of polypeptides synthesized de novo in a reticulocyte cell-free system under direction of rat lens 14*S* mRNA. (a) The big spot below the 20,000 mark is due to the formation of endogenous globin. For comparison, the patterns of α-crystallin polypeptides synthesized in the following homologous systems are shown; (b) epithelium of intact rat lens; (c) cortical fibers of intact rat lens; (d) rat lens cell-free system.

place in a variety of prokaryotic and eukaryotic cells. A list of eukaryotic proteins acetylated at the NH_2-terminus is shown in Table 1. We found that the acetylating machinery of heterologous protein-synthesizing systems acetylates the translation product of 14*S* lens mRNA. For instance, when this mRNA is injected into living oocytes, the completed αA_2 chains are acetylated at the NH_2-terminus (*32*). The same observation has been made after translation of the 14*S* mRNA in cell-free systems (*30, 37*). Thus, reticulocyte lysates or a wheat germ cell-free system programmed with α-crystallin mRNA are able to manufacture α-crystallin polypeptides acetylated at the NH_2-terminus undistinguishable from the native polypeptides.

We have isolated and partially purified an enzyme from calf lens, which is specific in that it catalyzes the attachment of the acetyl group at the N^{α} position of the NH_2-terminal amino acid residue, but not to the ϵ-amino group of lysine. Acetyl coenzyme A serves as donor of the acetyl group (*57*). Our experiments showed that a hexapeptide can serve as substrate, whereas tetrapeptides are not acetylated. The minimum length required for the substrate to become acetylated, therefore, seems to be a sequence of five to six amino acid residues.

The question has been asked: When



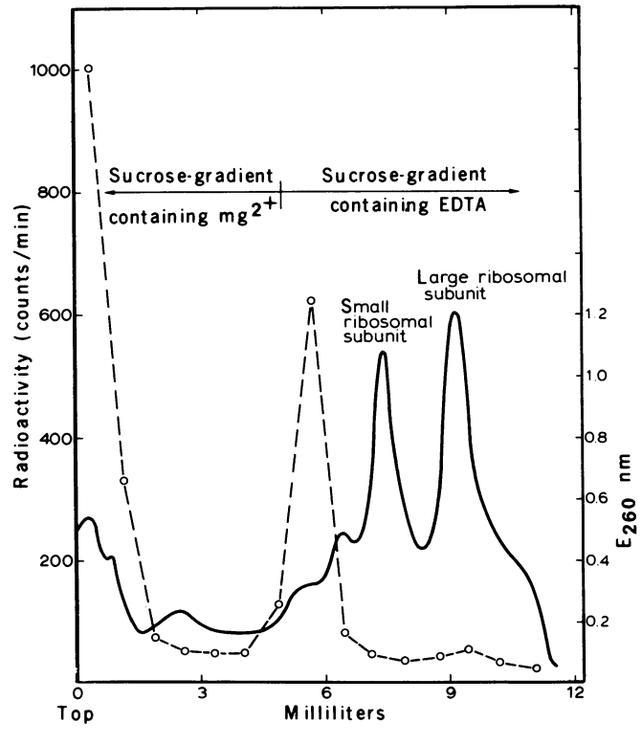


Fig. 9 (left). Kinetics of α-crystallin initiation in vitro with different [^{35}S]methionyl tRNA species. Abbreviations: *tRNA$_f^{Met}$*, initiator tRNA; *tRNA^{Met}*, tRNA species that donates methionine for internal positions in the polypeptide chains; *Ac*, acetyl; *f*, formyl. The unformylated Met-tRNA$_f^{Met}$ is a better initiator than the formylated one. Fig. 10 (right). Separation of nascent [^{35}S]methionine-labeled α-crystallin polypeptides from lens ribosomes by strip gradient centrifugation.

does NH_2-terminal acetylation take place? The lens cell-free system (*58*) can be used in order to determine the moment of acetylation. This system synthesizes the whole set of crystallins. [^{35}S]Met-tRNA$^{Met}_f$ and cofactors were added to a lens homogenate and incubated for 5 minutes. Thereafter, the polysomes were sedimented. The nascent polypeptide chains were isolated from the ribosomes after centrifugation through a special density gradient designated by us as "split gradient." The top half of such a gradient contains magnesium ions, whereas the bottom half is supplemented with EDTA (see Fig. 10). Polysomes carrying peptidyl-tRNA's dissociate into their subunits on reaching the EDTA-containing layer and the peptidyl-tRNA's are stripped off. The ribosomal subunits sediment toward the bottom, whereas peptidyl tRNA's remain in the interface. The peptidyl-tRNA's are isolated and treated with 0.1*M* NaOH at 37°C for 15 minutes. This treatment yields a mixture of tRNA's and peptides. The peptides can be fractionated according to size by gel filtration on Sephadex G-25. After digestion of the isolated peptide fractions with thermolysin, the resulting products were subjected to high-voltage electrophoresis. Thermolysin cleaves unblocked as well as α-crystallin chains acetylated at the NH_2-terminus that yield Met-Asp and acetyl-Met-Asp, respectively. The electropherogram of the thermolytic peptides from crystallin chains is shown in Fig. 11. The smallest chains contain only Met-Asp (panels 5 → 4). With increasing size of the peptides (panels 3 → 1) an increasing ratio between acetyl-Met-Asp and Met-Asp can be observed.

As all radioactivity recovered in the individual thermolytic peptides is donated exclusively by [^{35}S]Met-tRNA$^{Met}_f$, and as all α-crystallin chains bear NH_2-terminal methionine retained therefrom (*30*), the radioactivity behind Met-Asp (between 5 and 10 cm in Fig. 11, panels 4 and 5) can only be due to methionylpeptides derived from β- and γ-crystallin. It can be seen that this radioactivity disappears in panel 3 where label in the Ac-Met-Asp region starts to appear. This observation suggests that cleavage of NH_2-terminal methionine from newly synthesized β- and γ-crystallin chains coincides with the onset of, or just prior to, NH_2-terminal acetylation. Why methionine in α-crystallin and a few other proteins resists cleavage remains to be established.

The fact that not all proteins occurring in systems in which, apparently, the acetylating enzyme is present, carry an acetyl group at the NH_2-terminus suggests that the responsible enzyme recognizes some hitherto unknown structural feature of the polypeptide chain. In this connection, it should be mentioned that the proteins acetylated at the α-amino group of the NH_2-terminal residue, with only very rare exceptions, have Ala, Gly, Met, Ser, or Thr as the NH_2-terminal residue (compare Table 1).

However, since various proteins bear these amino acids at the NH_2-terminus in an unacetylated form, they cannot represent per se the recognition signal for the acetylating enzyme. At any rate, two pieces of evidence can be derived from our results: (i) in order to become acetylated, nascent polypeptide chains must have a length corresponding to about 25 amino acids; (ii) at least a peptide of an approximate length of five to six amino acid residues has to protrude from the ribosome, since this size is required for the substrate in order to enable the acetylating enzyme to exert its catalyzing activity. Nevertheless, from the data in Table 1, it is not easily apparent whether a certain regularity in the sequence of a number of amino acids near the NH_2-terminus is required for acetylation at the NH_2-terminus. The biological implication of acetylation of the NH_2-terminus is also obscure. Of course, proteins *N*-acetylated at the NH_2-terminal residue are protected against the attack of aminopeptidases. In the case of α-crystallin, a protein that has to survive for several decades, it is quite conceivable that this protection is needed, particularly since lens cells belong to the best sources for the isolation of leucine aminopeptidase (*59*). In contrast, some degradation of the COOH-terminal end seems permissible (*60*). This phenomenon is discussed below.

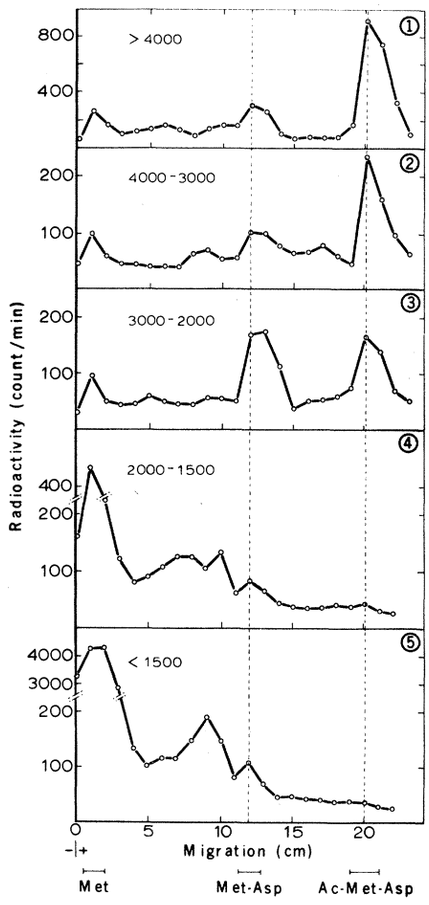
Table 1. Proteins of eukaryotic and viral origin, acetylated at the amino terminal.

Protein	NH_2-terminal
Basic protein (human myelin)	Ac-Ala-Ser-Gly-Lys-Arg
Basic A_1 protein (bovine myelin)	Ac-Ala-Ser-Ala-Gln-Lys
Cytochrome c (wheat)	Ac-Ala-Ser-Phe-Ser-Glu
Cytochrome c (box-elder)	Ac-Ala-Ser-Phe-Ala-Glu
Cytochrome c (ablutilon)	Ac-Ala-Ser-Phe-Gln-Glu
Cytochrome c (maidenhair tree)	Ac-Ala-Thr-Phe-Ser-Glu
Cytochrome c (castor)	Ac-Ala-Ser-Phe-Asx-Glx
Calcium-binding protein (hake)	Ac-Ala-Phe-Ala-Gly-Ile
Cucumber virus	Ac-Ala-Tyr-Asn-Pro-Ile
Carbonic anhydrase B (human)	Ac-Ala-Ser-Pro-Asp-Trp
Fructose 1,6-biphosphatase (rabbit muscle)	Ac-Ala-Asp-Lys-Ala-Pro
Myogen (carp)	Ac-Ala-Phe-Ala-Gly-Val
Pike parvalbumin III	Ac-Ala-Lys-Asp-Leu-Leu
Protein B_2 (wool keratin)	Ac-Ala-Cys-Cys-Ala-Pro
Protein B_{2a} (wool keratin)	Ac-Ala-Cys-Cys-Ser-Thr
Protein B_3 (wool keratin)	Ac-Ala-Cys-Cys-Ala-Arg
Bovine erythrocyte superoxide dismutase	Ac-Ala-Thr-Lys-Ala-Val
Apoferritin (horse)	Ac-Ser-Ser-Gln-Ile-Arg
Alcohol dehydrogenase (horse)	Ac-Ser-Thr-Ala-Gly-Lys
Alfalfa mosaic virus coat protein	Ac-Ser-Ser-Ser-Gln-Lys
Carbonic anhydrase (human)	Ac-Ser-His-His-Trp-Gly
Glyceraldehyde-3-phosphate dehydrogenase (lobster)	Ac-Ser-Lys-Ile-Gly-Ile
Hemoglobin α (carp)	Ac-Ser-Leu-Ser-Asp-Lys
Histone IV (bovine)	Ac-Ser-Gly-Arg-Gly-Lys
Histone I (bovine)	Ac-Ser-Glu-Ala-Pro-Ala
Myoglobin (mollusc *Aplysia limacia*)	Ac-Ser-Leu-Ser-Ala-Ala
Luteinizing hormone A-subunit (ovine)	Ac-Ser-Arg-Gly-Pro-Leu
α-MSH (melanotropin)	Ac-Ser-Tyr-Ser-Met-Glu
Parvalbumin (frog muscle)	Ac-Ser-Ile-Thr-Asp-Ile
TMV coat protein (vulgara)	Ac-Ser-Tyr-Ser-Ile-Thr
Cytochrome c (human)	Ac-Gly-Asp-Val-Glu-Lys
Hemoglobin, fetal (human)	Ac-Gly-His-Phe-Thr-Glu
Ovalbumin (chicken)	Ac-Gly-Ser-Gly-Ile-Ala
Troponin I (rabbit)	Ac-Gly-Asp-Glu-Glu-Lys
Actin (rabbit)	Ac-Asp-Glu-Thr-Glu-Asp
Lactate dehydrogenase M_4 (dogfish)	Ac-Thr-Ala-Leu-Lys-Asp
Fibrinopeptide (bovine)	Ac-Thr-Glu-Phe-Pro-Asp
α-Crystallin A_2 (bovine)	Ac-Met-Asp-Ile-Ala-Ile
α-Crystallin A_2 (rabbit)	Ac-Met-Asp-Val-Thr-Ile
α-Crystallin B_2 (bovine)	Ac-Met-Asp-Ile-Ala-Ile
Porcine adenylate kinase (skeletal muscle)	Ac-Met-Glu-Glu-Lys-Leu
Troponin	Ac-Met-Asp-Asp-Ile-Tyr

The Assembly of α-Crystallin

α-Crystallin is found in both distinct lens cell types, the epithelial cells and the fiberlike cells. Although in the epithelium the α-crystallin consists only of αA_2 and αB_2 [like de novo synthesized α-crystallin in the lens cell-free system derived from cortical fibers (*58*)], it is always found as an aggregation product of its subunits. The quantitative increase of αA_1 and αB_1 in the fiberlike cells has been related to the process of cellular growth and differentiation (*61*).

The isolated α-crystallin can be dissociated into its subunits by the addition of 6*M* to 8*M* urea (*62*) or 5*M* guanidine-hydrochloride (*63*). After removal of the perturbant by dialysis, reassociation of the polypeptides takes place. Despite intensive studies on the reaggregation phenomenon (*62–64*), virtually nothing is known about the underlying mechanism. Both α-crystallin and the β-crystallins consist of different polypeptide chains held together by hydrophobic linkages and hydrogen bonds (compare Fig. 4). Since the monomeric chains of α- and β-crystallins can form hybrids in vitro, one may ask which regulatory mechanism is operative that ensures the formation of the correct polymeric protein after synthesis and release of the primary chains. One possibility would be that, in some stages of the cell life, only one of the crystallins (for example, α-crystallin) is synthesized, while in other periods β-crystallin is produced exclusively. This assumption is unlikely in view of the fact that short-term labeling of whole lenses, as well as incubation of the lens cell-free system with radioactive amino acids, results in the synthesis of both α- and β-crystallin aggregates. Therefore, an alternative explanation that can be reached by comparison of experiments with other proteins seems more reasonable. For bovine thyrotropin (*65*) and ovine pituitary interstitial cell–stimulating hormone (*66*), it has been reported that there exists a difference in order between the folding of the peptide chains and their association. A similar situation may exist in the case of the crystallin chain assembly. If the proper folding of the primary crystallin chains is a first-order reaction, whereas the interchain interaction is of higher order, then correct sorting out will be achieved. In order to verify this interpretation, we carried out a series of experiments with all of the water-soluble crystallins dissociated in 7*M* urea (*67*). The results showed that correct renaturation can only be achieved when dissociation and removal of urea (reassociation of the polypeptide chains) was carried out at rather high dilution (2 mg/ml) (Fig. 12). In that case, from a mixture of at least ten different kinds of polypeptide chains, the various "native" high-molecular-weight aggregates are reconstituted. In contrast, at relatively high concentration (200 mg/ml) of the polypeptide chains, αβ hybrid formation was always observed. Therefore, it seems that a rather trivial regulatory mechanism for correct assembly of polymeric protein may be operative, at least in vitro, namely, the concentration of the composing polypeptide chains. Before this result is extrapolated to the in vivo



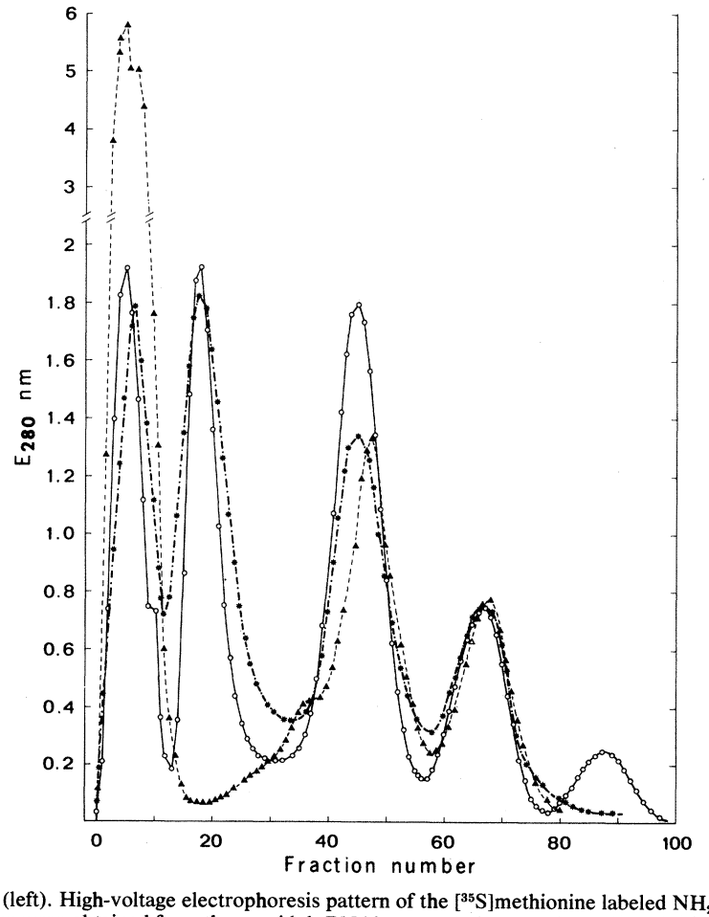


Fig. 11 (left). High-voltage electrophoresis pattern of the [^{35}S]methionine labeled NH_2-terminal dipeptides of newly synthesized α-crystallin. The peptides were obtained from the peptidyl-tRNA's separated on a strip gradient (Fig. 10). After gel filtration of the isolated peptides thermolytic cleavage of the separated fractions was applied. The molecular weight ranges of the peptides before enzymic digestion are indicated at the left upper part of the panels. Fig. 12 (right). Gel-filtration patterns of native and reassociated lens crystallins on Sephadex G200. o—o—o, native crystallins; ▲--▲--▲, crystallins reassociated at a protein concentration of 200 mg/ml; •--•--•, crystallins reassociated at a protein concentration of 2 mg/ml.

situation, it is necessary to rule out the possibility that crystallin polypeptide chains undergo proper folding while still being attached to the ribosome.

Aging of α-Crystallin

Interest in the process of aging of lens proteins arises not only from a general point of view, but also particularly from the unsolved problem concerning the visual impairment in senile cataract. In principle, any reliable information about changes caused by aging requires observation during extended periods of time. As far as aging of proteins is concerned, there is the general phenomenon of loss of material, which implies an imbalance between biosynthesis and breakdown. The vertebrate eye lens, however, forms an exception to that rule since the intracellular protein level is virtually maintained during the whole life span of the animal. Thus, the lens provides an especially useful system for the study of aging. Proteins present in the nucleus of the adult lens have been synthesized during fetal life. Hence any change of protein observed in this part of the lens or in the inner cortex reflects either post-synthetic alterations, or, less likely, a differing biosynthetic activity in the early stages of lens development.

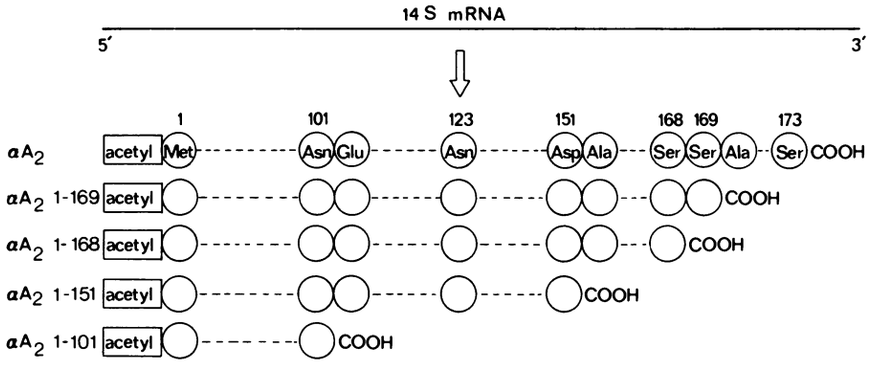


Fig. 13. Tentative aging scheme of the αA_2 chain, the primary translation product of 14S mRNA. The shortened chains A_2^{1-169} and A_2^{1-151} are already present in the embryonic stage. A_2^{1-151} is more abundant in adult nuclear α-crystallin than in embryonic α-crystallin. A_2^{1-101} is not detectable in embryonic α-crystallin. A similar sequential degradation takes place in the αA_1 chain which arises by transition of the *Asn* to the *Asp* residue in position 123 (deamidation). The distance between αA_2 and αA_1 on polyacrylamide gels (Fig. 4), however, suggests that still another *Asn* or *Gln* is replaced by *Asp* or *Glu* residues, respectively.

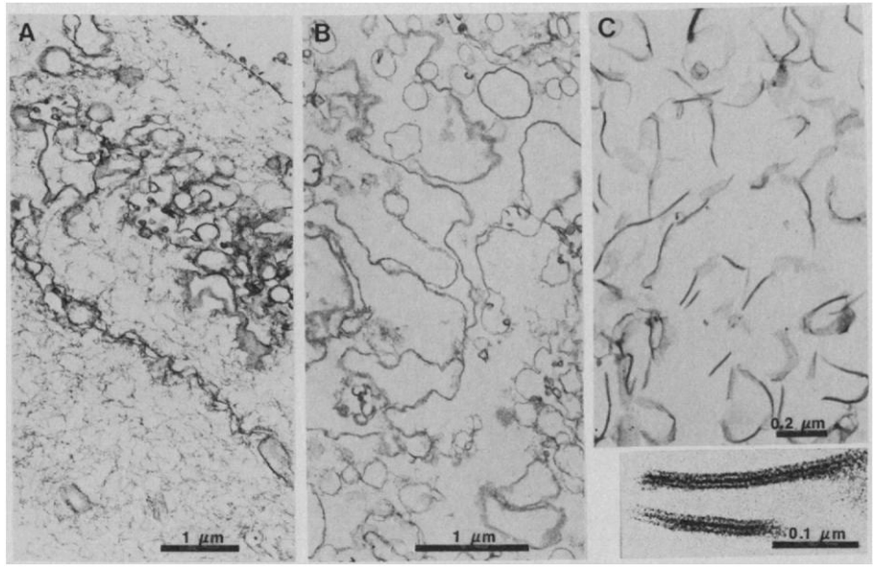


Fig. 14. Electron micrograph of thin sections of lens membrane fractions. (A) The crude water-insoluble preparation consisting of fiber ghosts. Plasma membrane regional specializations can be identified as forming either interdigitations or intercellular junctions. Remnants of polyribosomes and the filamentous network are found in close association with the plasma membrane. (B) Purified plasma membrane preparation. (C) Isolated communicating junctions obtained after sarkosyl treatment and equilibrium density gradient centrifugation of purified membranes. Note the typical pentalayered feature of the junctional membrane element (inset).

The lens protein α-crystallin is subject at least to three age-related and irreversible processes, namely (i) the formation of high-molecular-weight aggregates; (ii) progressive deamidation of the primary polypeptide chains; and (iii) defined degradation of polypeptides starting from the COOH-terminal end. An experimental approach to the degradation problem became possible after the complete primary structure of the major α-crystallin chain (αA_2) was available (*20, 21*). The αA_2 chain, consisting of 173 amino acids (compare Fig. 7), gradually gives rise to the αA_1 chain, which is absent in fetal calf lens (*19, 23*) and has the same amino acid composition, except that two Asn residues have been converted into Asp (Fig. 13). The finding that αA_1 is already present in the lenses of very young calves suggests that the deamidation process might be a maturation step rather than a proper phenomenon of aging (*68*), at least as far as lens tissue is concerned. As deamidation can artificially be induced in vitro by storing purified αA_2 in solution, the transition to αA_1 seems not to be an enzymatic process (*69*). The first observation that led us to the conclusion that aging of α-crystallin is accompanied by COOH-terminal degradation was the finding of a minor tryptic peptide which differed from the COOH-terminal αA_2 tryptic peptide Glu-Glu-Lys-Pro-Ser-Ser-Ala-Pro-Ser-Ser in that the five terminal amino acids were absent (*60*). Further detection of shortened αA chains lacking constant numbers of amino acid residues from the COOH-terminal end (*70*) provided the basis for the aging scheme of the αA chains shown in Fig. 13. Degradation from the COOH-terminal end has also been observed for the αB chains and for β-crystallin polypeptides (*71*).

The Lens Membranes

Although α-crystallin is defined as the major water-soluble structural protein of the mammalian eye lens, a water-insoluble fraction is always isolated together with the plasma membranes of the lens. This has led to the question of whether there exists a functional interaction between α-crystallin and the membranes (*10, 72*). Before that question can be answered, it is necessary to know something about the composition and structure of lens membranes.

I have mentioned that during differentiation of the lens epithelium into fiberlike cells most of the cellular organ-

elles disappear, with exception of the polyribosomes and the plasma membranes. Since the fibers have a very high ratio of surface to volume, these cells are an extremely good source for the isolation of plasma membranes and hence may provide a suitable model system for studying the architecture of cell membranes in general.

Lens plasma membranes are characterized by regional specializations. Some of these specializations are involved in essential membrane functions—for instance, the regulation of transport, permeability, and cell-to-cell communication. Moreover, lens plasma membranes appear to be closely associated with cytoplasmic structures, such as microfilaments and microtubules that have an important role in maintaining cell shape and the physical parameters upon which lens accommodation may rely.

The isolation of lens plasma membranes has been achieved essentially by two procedures. The first one (*9, 10*) is based on a gentle homogenization of the decapsulated lens in 1 m*M* sodium bicarbonate, followed by centrifugation at low speed and repeated washings in order to remove water-soluble lens components. The resulting pellet is a rather crude membrane preparation which still contains other membranous cell organelles and remainders of polyribosomes (Fig. 14A). Further purification is carried out by suspending the pellet in a sucrose solution to a final density of 1.22 g/cm^3. This suspension is used as bottom layer of a discontinuous sucrose gradient consisting of layers with densities of 1.20, 1.18, 1.16, and 1.14 g/cm^3, respectively. After centrifugation at 100,000*g* for 150 minutes, the membranes float at the interface of the layers with densities of 1.16 and 1.14 g/cm^3. An electron micrograph of such a purified preparation is shown in Fig. 14B. The extensive membranous profiles are interconnected by pentalaminar structures representative for junctions (Fig. 14C).

The second isolation procedure is based upon the solubility properties of the various membranous constituents. Decapsulated lenses are dissolved either by homogenization or by stirring for several hours in a slightly alkaline buffer to extract the water-soluble crystallins. After centrifugation at 40,000*g* for 15 minutes, the sedimented material is extracted with 7*M* urea. The extract is then centrifuged at 100,000*g* for 15 minutes; the supernatant is the urea-soluble lens protein; the sediment, which is insoluble in urea, can be dissolved in SDS and is called the urea-insoluble lens protein (*73*). The latter pellet contains rather impure membranes, whereas by the first procedure a membrane fraction is isolated which is representative for all regional specializations existing in situ, as, for instance, intercellular junctions.

The Lens Membrane Proteins

The protein pattern of isolated lens fiber membranes is characterized by a number of components that occur in different proportions (Fig. 15F). With exception of an intensively stained band with a molecular weight of 26,000, they are all located on SDS gels in the molecular weight region above 32,000. This condition allows their separation from the lens crystallins which migrate in the molecular weight region of 18,000 to 32,000. Strikingly, even after repeated washing in low ionic strength buffer, mild proteolytic digestion, and subsequent elution with 7*M* urea, crystallin polypeptides are still detectable in the lens membrane protein profile on SDS-polyacrylamide gels (*9, 10*) (compare Fig. 15F). Therefore, it is not unlikely that crystallins, at least in part, represent integral membrane constituents (*74*). This raised the question as to the conformation of the so-called water-soluble crystallins in the lens. In view of lens function it is hard to imagine that the fibers are just sacs filled with an amorphous mass of protein. On the contrary, there is growing evidence provided by high-resolution electron microscopy (*67, 75*) that cytoplasmic lens protein exists in an ordered framework of chainlike structures (compare Fig. 16). The crystallin polypeptides thus may form a stable association with actin-like microfilaments in situ. We have observed that microfilaments are directly associated with the plasma membrane inner surface (*74*). The molecular nature of this direct structural contact, however, is still unknown. In this connection, it must be mentioned that the role of actin has already been established in other cellular systems (*76*). Cytoplasmic microfilaments formed by actin-like protein have, for example, been identified in nonmuscular cell types.

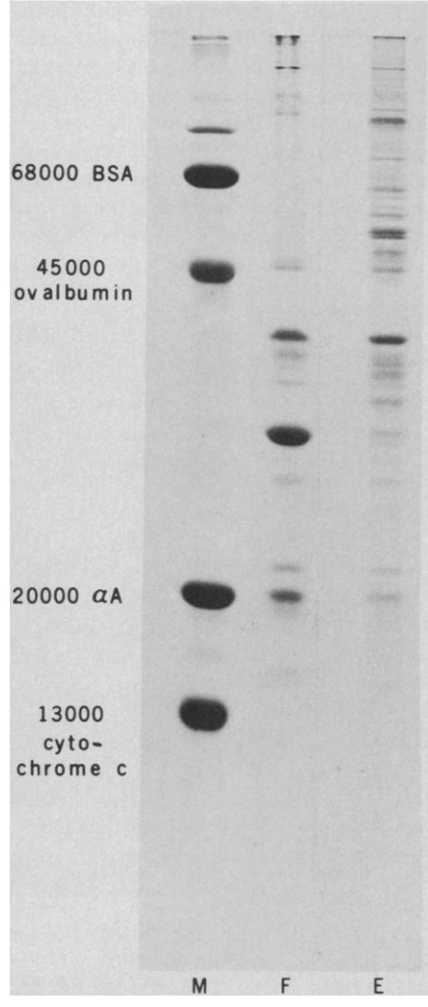


Fig. 15. Sodium dodecyl sulfate gel electrophoresis pattern of purified lens plasma membrane proteins. Membrane proteins from lens fiber (*F*); membrane proteins from epithelium (*E*). The marker proteins (*M*): bovine serum albumin (*BSA*), ovalbumin, α crystallin A_2 chain (αA), and cytochrome c, served as standards for the calculation of approximate molecular weights.

Another interesting aspect of the study on lens plasma membranes is that related to the chemical nature and structural feature of the junctions connecting lens cells throughout the entire thickness of the organ (*77*). The communicating junctions which connect the epithelial layer (where adenosine triphosphatase is located) to fibers, and those that link fibers to fibers favor translenticular maintenance of electrochemical gradients that are essential for a correct balance of the intra- and intercellular milieu. As in other tissues, the junctions of the lens fibers are characterized by their insolubility in various detergents and by a rather simple protein pattern (*10*). In the lens fiber plasma membrane profile, the two typical polypeptides that have molecular weights of 26,000 and 34,000, respectively, become predominant in the fraction that consists mainly of the communicating junctions. The most striking difference between the protein patterns of lens plasma membranes derived from epithelium and fibers, respectively, concerns the accumulation of the 26,000-molecular-weight polypeptide in the fiber membranes, while there is no obvious difference in the distribution of the other polypeptides (*78*). This means that differentiation of epithelium into fibers,

which is visually characterized by the feature of cell elongation (*79*), is accompanied by the formation of a specific membrane protein (Fig. 15, F and E). It should also be mentioned that electron microscopy (*80*) revealed an essential morphological aspect of the membranes during differentiation of epithelium into fiberlike cells, namely, the progressive accumulation of particles within the hydrophobic core of the membrane leaflets in areas of cell-to-cell contact (Fig. 17) (*81*).

Noncrystallin Messenger RNA

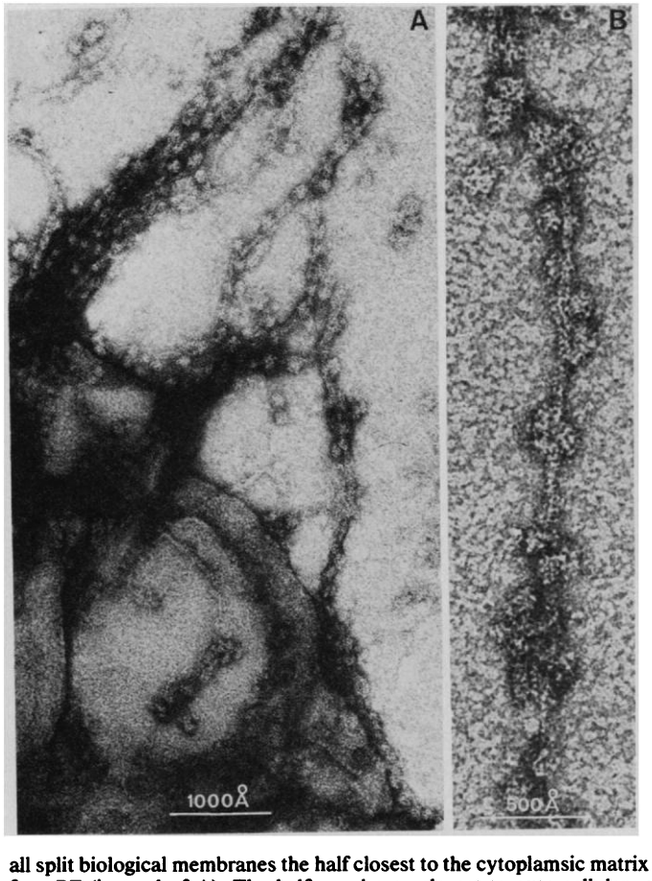
It remains to be elucidated whether membrane formation and the assembly of junctional devices during lens cell elongation relies on de novo synthesis of individual membrane constituents rather than on lateral displacement of preexisting structures.

We have pointed out (*82*) that the isolation and characterization of noncrystallin mRNA's are relevant for the study of lens plasma membrane synthesis. The experimental approach for such investigations is facilitated by the fact that on SDS-polyacrylamide gels crystallin subunits migrate with an electrophoretic mobility different from that of the membrane polypeptides (*9, 10*).

As a result of previous studies, it was found that the lens cell-free system is able to initiate, elongate, terminate, release, and assemble polypeptides that are indistinguishable from isolated native crystallins (*58*). Since, however, the product analysis was carried out on 7*M* urea–polyacrylamide gels, specific intrinsic membrane proteins, if synthesized, must have escaped detection because of their insolubility in urea. Reinvestigation of the products synthesized de novo in the cell-free system by analysis on SDS gels and subsequent scintillation autoradiography (*83*) revealed the formation of polypeptides which on coelectrophoresis with membrane-specific polypeptides traveled with them (*82*). Moreover, it appeared that also reticulocyte lysates supplemented with polysomes that were isolated from the outer lens cortex synthesized the same set of membrane-specific components (*82, 84*). Furthermore, we were able to provide direct evidence, by immunoprecipitation of the newly synthesized polypeptides with a specific antiserum directed against membrane polypeptides, that the 34,000-molecular-weight component was synthesized in the heterologous system (*78*). These findings showed that mRNA encoding the membrane proteins is present in the lens cortex. We noticed that the translational activity of these mRNA's vanished after their purification on oligodeoxythymidylate-cellulose columns. Eventually this could be overcome by thorough deproteinization of the preparation (*84*) and a slight modification of the commonly used affinity chromatography procedure of Aviv and Leder (*85*). Furthermore, we have evidence that especially the noncrystallin lens mRNA's have a tendency to form complexes with ribosomal RNA. A similar phenomenon has been suggested by Kabat for globin mRNA (*86*).

Concluding Remarks

The various aspects of biochemical properties of the vertebrate lens that I have presented can be considered as bits of information useful for general studies on the biosynthesis, postsynthetic alterations, and assembly of proteins. For a better understanding of the molecular architecture and function of the lens as a



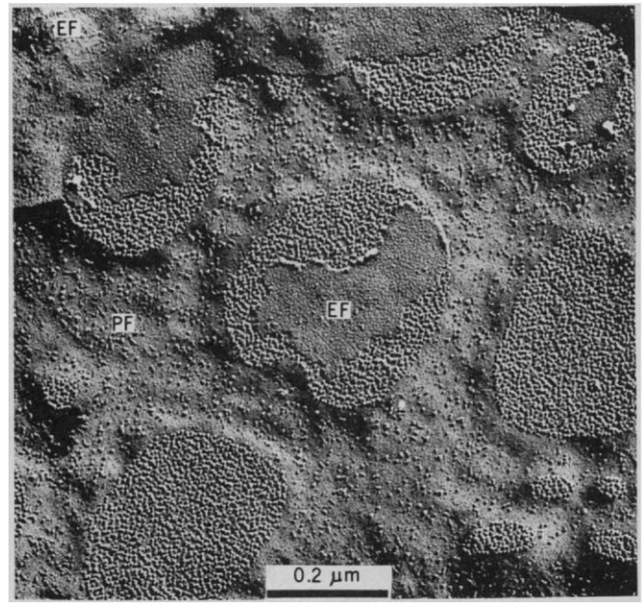


Fig. 16 (left). Isolated lens fiber plasma membranes negatively stained with uranyl formate after repeated washing in low ionic strength buffer. (A) The plasma membrane sheets appear to be associated with microfilaments and with molecules resembling crystallin. (B) High magnification of a similar preparation. Fig. 17 (right). Freeze-fracture replica of communicating junctions between elongating lens fibers. Multiple particulate domains, suggesting that during junctional assembly smaller clusters of intramembranous particles merge into more extensive arrays, are visible. The nomenclature for freeze-fracturing and etching has been revised (*87*). It has been proposed that for all split biological membranes the half closest to the cytoplamsic matrix should be labeled the protoplasmic half (*P*) and its corresponding fracture face *PF* (instead of A). The half-membrane closest to extracellular, exoplasmic, or endoplasmic spaces should be labeled E and its fracture *EF* (instead of B).

unitary biological system, the following remarks appear pertinent.

In contrast with other organs the lenticular mass is composed of virtually identical cells (the fibers) derived from a single cell type (the epithelium). This multicellular system synthesizes a limited number of well-defined proteins. Moreover, it represents an evolutionary product that is able to carry out by itself the whole set of metabolic functions, such as adenosine triphosphate synthesis (mainly through the glycolytic pathway) and protein, nucleic acid, and lipid formation as required for its ontogenic development and maintenance. During its entire life span the lens is the site of production, assembly, and postsynthetic processing of both structural protein and polypeptides that will be inserted in highly specialized membrane regions. Three characteristics make lens cells suitable for model studies:

1) The occurrence of a stable messenger population which facilitates the exploration of cell-free systems for de novo synthesis of crystallins and membrane proteins.

2) A burst of membrane formation during cell-elongation which offers a unique possibility to investigate membrane assembly.

3) An easily identifiable protein profile of the purified plasma membranes which enables one to discriminate between the crystallins, the specific membrane polypeptides, and cytoskeletal constituents (*88*).

Moreover, comparative analyses of the primary structure of the lens protein α-crystallin with its highly conservative nature among the species examined so far (*89*), will provide useful information to broaden our phylogenetic knowledge. From the data now available, one may conclude that refraction by the lens of incident light does not allow dramatic changes of the amino acid sequence of the major lenticular protein.

As far as the aging of α-crystallin is concerned, I conclude that while the polymeric protein is growing with respect to molecular weight (HM-α-crystallin), a portion of the composing subunits is, in contrast, subject to spontaneous degradation from the COOH-terminus.

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48. Abbreviations: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Cys, cysteine; Glu, glutamic acid; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine.
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89. α-Crystallin was isolated from lenses of 27 vertebrate species [W. W. de Jong, E. C. Terwindt, G. Groenewoud, *Comp. Biochem. Physiol.* **55B**, 49 (1976)].
90. I am grateful to Professor E. L. Benedetti (Laboratoire de Microscopie Electronique, Institut de Biologie Moléculaire, Paris) for his helpful comments and for the electron microscopic illustrations shown here. I thank Dr. A. J. M. Berns for valuable discussions. The indispensable help of a group of medical students who have isolated almost 1 million calf lenses since 1966, is gratefully acknowledged. Part of the investigations described here have been supported by the Netherlands Foundation for Chemical Research (S.O.N.) and by the Netherlands Organization for Pure Research (Z.W.O.). I also thank M. C. Potjens for her assistance in preparing the manuscript.

NEWS AND COMMENT

Central Crime Computer Project Draws Mixed Reviews

After committing a crime in Ohio, an offender from California, for example, may flee to Florida, having profitably disposed of his stolen goods in still another State. With all the marvels of modern transportation, all this can and does occur while the police pore over the fresh traces at the crime scene.

To solve crime in the space-age tempo of today's society demands that law enforcement officers have available immediately the facts of crime whenever or wherever they are likely to confront suspected elements of it.

—CLARENCE M. KELLEY, director, Federal Bureau of Investigation

The Federal Bureau of Investigation (FBI) is currently debating the future of a computerized criminal information system designed in the late 1960's to help police keep up with the modern, mobile criminal, who, as the director of the FBI wrote in the above passage in 1974, can be moving on across the country "while police pore over the fresh traces at the crime scene."

The system is called the Computerized Criminal History (CCH) and includes about 1 million people who have been arrested in recent years for federal or state offenses. The CCH is part of a larger system known as the National Crime Information Center (NCIC), which includes, besides CCH, files on stolen property, vehicles, license tags, boats, securities, and on missing and wanted persons.

Most of the publicity about the CCH file has concerned its civil liberties implications, and at least one state, Massachusetts, has declined to participate in CCH on civil liberties grounds. But CCH's future in its present form is in doubt, primarily because of less-publicized, practical problems which lessen its utility as a law enforcement aid.

It turns out that, whereas 48 states can have access to the CCH files, less than a dozen states have been turning over their records to the central Washington file principally because of the cost, time, and trouble. Hence, the CCH includes only 1 million of the 21 million persons with recent records of arrest for serious crimes. Not only is it incomplete, the CCH system lacks the one capability that would make it a vital tool for police, courts, and corrections officials—namely, the capability for virtually error-free identification of suspects through automated, instantaneous matching of fingerprints. For this service, the authorities still use the FBI Identification Division's file of 21 million sets of prints, which they reach through the U.S. mails. And, while the mail does not exactly travel at "space age tempos," the resulting service at least has the important benefit of providing reliable information.

Citing these and other problems, Kelley wrote a secret memorandum to the Attorney General, Edward Levi, in April 1976, requesting him to terminate the CCH altogether. Neither Levi nor his successor have acted on the request. However, the Carter Administration and the Congress are studying a controversial proposal which could be a step toward decentralizing the CCH and redistributing control of the criminal history files among the states.

Like many other incursions of new technology into the law enforcement sphere, the CCH grew out of research performed under the auspices of the Law Enforcement Assistance Administration (LEAA), the Justice Department's research arm which was lavishly supported