*Review–Hypothesis*

# Proteolytic control over topogenesis of membrane proteins

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## 1. INTRODUCTION

According to present-day concepts, to give rise to receptors, enzymic complexes and other membrane multisubunit assemblies, many polypeptides undergo intricate conversions on their way from the site of synthesis to the place of destination in the cell (see, e.g. [1]). These conversions include proteolytic processing, primary glycosylation in the endoplasmic reticulum, final glycosylation in the Golgi apparatus, conformational maturation, intracellular transport, etc. The question arises as to how, as all this takes place, strictly definite stoichiometric relations between subunits are established in the cell. This paper presents data from which it follows that this is achieved not by rigorously coordinated synthesis of subunits but rather through elimination of unassembled or incorrectly compartmentalized polypeptides and complexes not integrated into functioning higher-order structures (supercomplexes).

## 2. ASSEMBLY OF THE MEMBRANE FORM OF IgM IN B CELLS

It is known that B cells at all differentiation stages synthesize heavy chains of IgM [2]. In pre-B cells the heavy chains ($\mu$) are not seen, the sole reason for this appearing to be the absence of light (L) chains. Indeed, $\mu$ chains are expressed in the surface IgM in hybrids formed between the L chain-lacking pre-B-like lymphoblastoid cells of the line Josh 4 and L chain-producing lymphoblastoid cells RPMI-8806 (IgG$\varkappa$) and 32a1 (IgA$\lambda$) [3]. A similar situation is found in resting B cells or in phenotypically close cells of the Daudi line which, in contrast to pre-B cells, synthesize L chains [4]. These data led to the suggestion that heavy chains not bound with the light ones undergo rapid degradation.

Having studied the degradation kinetics of $\mu$ chains in Daudi cells, Dulis et al. [4] found that the membrane form of the heavy chains ($\mu^m$) consists of a heterogeneous population comprising rapidly ($\tau_{1/2}$ = 1.4 h) and slowly degrading ($\tau_{1/2}$ = 13 h) fractions. Only rapidly degrading $\mu^m$ chains are seen in the presence of tunicamycin, which impairs IgM assembly.

It follows from these and a number of other data presented by Dulis et al. [4] that the existence of the stable form of $\mu^m$ is a corollary to the formation of complete IgM and its expression on the plasma membrane.

It is known that in the course of formation of surface IgM the $\mu^m$ chains undergo primary glycosylation, most probably cotranslationally, then covalently bind to the L chains to make a complex $\mu_2^mL_2$, and are finally glycosylated in the Golgi complex [4]. Mature IgM is incorporated into the cell plasma membrane, leaving the zone of action of intracellular proteolytic systems. The question is at which of these steps the chains acquire resistance to proteolysis.

Using tunicamycin and the carboxyl ionophores monensin and nigericin, Dulis and co-workers [4,5] showed that stabilization of the $\mu^m$ chains oc-

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curs after the primary glycosylation step but before the final glycosylation and transfer to the plasma membrane. This gave grounds for suggesting that the $\mu^m$ chains are stabilized simply owing to their binding with the L chains.

This suggestion was confirmed by trypsinization of Daudi cell homogenates containing various associated forms of $\mu^m$ (i.e. $\mu_2L_2$, $\mu_2L$, $\mu_2$, and $\mu L$). Such treatment led to an increase in relative content of $\mu^m$ chains in the $\mu_2L_2$ form [5].

Thus, expression of the heavy $\mu^m$ chains of IgM in B cells depends on the presence of the light chains. In the absence of their partners the heavy chains are subject to exceptionally rapid proteolysis. Suffice it to recall that the half-life of $\mu^m$ chains not integrated into IgM is 1.4 h, whereas that of integrated chains is 13 h and the total cell protein of Daudi cells has a half-life of 32 h [4]. In other words, in B cells the expression of heavy chains of surface IgM is under efficient post-translational control: on the one hand, a powerful proteolytic system is in operation, and on the other, integration of $\mu^m$ chains into assemblies with L chains renders them inaccessible to proteinases.

## 3. INCORPORATION OF $\alpha$-SUBUNITS INTO ACETYLCHOLINE RECEPTORS

Topogenesis of $\alpha$-subunits of acetylcholine receptors comprises synthesis of the polypeptide chain on polysomes associated with the endoplasmic reticular membrane, cotranslational primary glycosylation of the polypeptide, its conformational maturation, integration with other subunits into the receptor, and finally transfer of the receptor from the Golgi complex to the cytoplasmic membrane [6].

As shown by pulse-chase experiments [7], in mouse line BC3H-1 cells about one quarter of the total amount of newly synthesized subunits are incorporated into the complex able to interact with $\alpha$-bungarotoxin, while more than three quarters are quickly degraded. The half-life of free $\alpha$-subunits was estimated at 0.1–0.5 h [7], whereas the half-life of the acetylcholine receptor is 8 h [8].

It remains to be elucidated which step in the topogenesis of subunits gives rise to their stable form. Primary glycosylation is known to increase the half-life of subunit precursors no more than 3-fold and their breakdown is accelerated to the same degree in the presence of tunicamycin. At the same time tunicamycin decreases the effectiveness of receptor assembly from 30 to 5%, i.e. 6-fold. Merlie et al. [7] suppose that this is caused not by degradation of free non-glycosylated precursors of $\alpha$-subunits but by interruption of the transport of $\alpha$-subunits into the Golgi complex where they are bound with other subunits of the acetylcholine receptor. In other words, the critical event in the stabilization of $\alpha$-subunits appears to be their transfer to the Golgi complex. Whether this is due to their interaction with other receptor components or to their withdrawal from the zone of action of the cell catabolic system remains unresolved.

## 4. INCORPORATION OF ANKYRIN AND SPECTRINS INTO THE MEMBRANE SKELETON

The membrane skeleton of erythroids is an assembly of several types of proteins. The skeleton as such is composed of a two-subunit protein, spectrin, which accounts for 75% of the total mass of the complex. The acceptor for spectrin is a surface membrane protein, ankyrin, binding to the transmembrane anion carrier.

As shown by Moon and Lazarides [9], a considerable proportion of ankyrin and spectrins after completion of their synthesis is not incorporated into the skeleton but remains in the cytosol and is rapidly catabolized. With newly synthesized $\alpha$-spectrin, its content in soluble form after a pulse of [$^{35}$S]methionine is more than twice that of the membrane-bound form. The initial contents of labeled $\beta$-spectrin and ankyrin in the cytosol are about 70% of that in the membrane skeleton. All these proteins in the membrane are not appreciably degraded in at least 4 h, though the half-lives of $\beta$-spectrin and ankyrin in solution do not exceed 0.5 h.

Thus, the stoichiometry between polypeptides in the cell is set posttranslationally through rapid elimination of unassembled polypeptide chains. It should be noted that ankyrin forms a complex with spectrin in solution [10]. However, the experimental data mentioned above demonstrate that such an interaction does not give rise to stable assemblies. Since the affinity of ankyrin for the anion carrier is an order of magnitude higher than for spectrin

[11], it can be thought that initially ankyrin binds quickly to the carrier and then accepts spectrin, thereby forming the membrane skeleton whose components are rendered inaccessible for the cell catabolite system. According to Moon and Lazarides [9], this is the gist of the "preventive mechanism that the cell has to ensure the accumulation of the assembled complexes only at the correct cytoplasmic site".

## 5. FORMATION OF THE MITOCHONDRIAL RESPIRATORY CHAIN

The mitochondrial respiratory chain comprises several interacting oligoenzyme complexes built of polypeptides of cytoplasmic or both cytoplasmic and mitochondrial origin. The number of such polypeptides per complex varies from 6–7 to 16 (see references in [12]).

Polypeptides synthesized in the cytosol as precursors with amino-terminal extensions are received by mitochondria, transported in an energy-dependent way across mitochondrial membranes and proteolytically processed on the M and C sides of the inner mitochondrial membrane; sometimes precursors are processed in several steps (see references in [13,14]). As to polypeptides of mitochondrial origin, some are synthesized as larger precursors and processed whereas others have no precursors (see references in [12]).

Mature polypeptides (some of them after covalent or noncovalent binding of prosthetic groups) gradually 'find' their partners in the membrane and unite into complexes. Attempts have been made to estimate the approximate sizes of free subunit pools for complexes [15,16].

It was as far back as 1976 that we published our preliminary data [17] from which it followed that assembly of the respiratory chain in yeast is accompanied by formation of surplus amounts of cytochromes *b*, *c*, $c_1$ and $aa_3$ which are not needed to maintain the mitochondrial respiration at a constant level and are gradually eliminated with the participation of some intracellular proteinases without a concomitant decrease in the cell respiration rate. This elimination could be hindered with proteinase inhibitors administered to the cells in such a way that they did not appreciably affect culture growth.

Detailed studies of this phenomenon [18] showed that the 'excess' cytochromes most likely enter into the composition of excess respiratory complexes, which display enzymic activity after mild membrane solubilization but do not contribute to the respiratory activity of the mitochondrial population. Some indirect data suggested that the nonfunctioning complexes were incorporated incorrectly into the membrane and therefore could not interact efficiently with their partners in the respiratory chain [18]. The molecular basis for selection of operative respiratory complexes ('selection by a performance criterion') has been considered elsewhere [12].

It is easy to see that while the preceding sections concerned the rejection of individual subunits of membrane protein complexes, in this case we are dealing with degradation of whole oligoenzyme complexes that failed to enter a more highly organized functional unit, or 'supercomplex'. Unfortunately, this process has not yet been elucidated in detail. In particular, it is not clear whether it involves the endogenous mitochondrial [19,20] or the cytoplasmic [21] proteolytic system, or both.

The proteolytic control of mitochondrial assembly appears to be carried out at several levels. According to a number of authors [22–24], precursors of mitochondrial proteins synthesized in the cytosol are rapidly broken down (with a half-life of 5–10 min) if their transport into mitochondria is impaired. It is also known that the half-life of polypeptides synthesized in mitochondria when cytoplasmic translation is blocked (i.e. in deficiency of cytoplasmically made partners) may be only 15–20 min [25]. Degradation of these polypeptides involves specific mitochondrial proteinases [26].

Thus, the cell possesses proteolytic systems capable of efficiently controlling the assembly of the mitochondrial respiratory chain through elimination of incorrectly compartmentalized and unassembled polypeptides.

The same seems also to hold true for the assembly of membrane complexes in chloroplasts. For example, *hcf*-3*, a maize nuclear mutant lacking photosystem II activity, has been shown to have an accelerated turnover of chloroplast-encoded 48-kDa and 34.5-kDa polypeptides [27]. These polypeptides cannot form photosystem II reaction centers because of the lack of nucleus-encoded partners and appear to be degraded either

before, during, or immediately after their import from stroma to the grana.

Similarly, the light-harvesting chlorophyll *a/b*-protein undergoes rapid turnover in the thylakoid membrane when chlorophyll *a* and chlorophyll *b* are not synthesized [28].

It has been found also [29] that cytoplasmically synthesized subunits $\gamma$ and $\delta$ of the $CF_1$ chloroplast coupling factor of photophosphorylation are degraded rapidly and selectively in rye leaves under conditions when chloroplast-encoded subunits $\alpha, \beta$ and $\epsilon$ are not produced. Rapid degradation of unassembled subunits was considered a mechanism controlling 'a constant stoichiometry and apparently synchronous development of $CF_1$ subunits'.

## 6. CONCLUSION

As follows from the above, 'hypersynthesis' of components of membrane multisubunit complexes and 'overproduction' of whole complexes with subsequent elimination of unassembled and nonfunctioning material is a widely encountered biological phenomenon. It most probably has its roots in the complexity and considerable duration of polypeptide topogenesis [30]. Thus, synthesis of the already mentioned $\alpha$-subunit of the acetylcholine receptor lasts for about 1 min, whereas its subsequent maturation and binding with other subunits take some 2 h [6] during which the subunit is unprotected and may be a target for proteinases.

Table 1 lists the half-lives of some polypeptides in free form and in membrane complexes, and pro-

Table 1

Accelerated degradation of unassembled and incorrectly compartmentalized polypeptides or nonfunctioning complexes

| Polypeptides | Organism | Half-life | Proportion of rapidly degraded newly synthesized polypeptides (%) | Ref. |
|---|---|---|---|---|
| Acetylcholine receptor | mouse cell | | | |
| Free $\alpha$-subunit | line BC3H-1 | 0.5 h | 75 | [7] |
| $\alpha$-Subunit in receptor | | 8 h | | |
| Surface IgM | Daudi cell | | | |
| Free[a] $\mu^m$ chain | line | 1.4 h | 80 | [4] |
| $\mu_m$ chain in IgM | | 13 h | | |
| Membrane skeleton | avian erythroid | | | |
| Ankyrin, soluble | cells | about 0.5 h | 40 | [9] |
| Ankyrin, membrane-bound | | >4 h | | |
| $\beta$-Spectrin, soluble | | about 0.5 h | 40 | |
| $\beta$-Spectrin, membrane-bound | | >4 h | | |
| Respiratory chain of mitochondria | yeast | | | |
| Cytoplasmically made polypeptides not imported into mitochondria | | 5–10 min | | [22,24] |
| Mitochondrially made polypeptides not integrated into complexes (?) | | 15–20 min | | [25] |
| Nonfunctioning respiratory complexes (succinate:ubiquinone reductase; ubiquinol:cytochrome *c* reductase, cytochrome *c* oxidase) | | 2–3 h | 25–30 | [18] |

[a] Not integrated into IgM

portions of polypeptides undergoing breakdown in the course of their topogenesis. In this context certain physiological meaning is conferred on the data on the proteolysis of so-called rapidly labeled proteins which are degraded at a rate an order of magnitude higher than that of the general protein catabolism in the cell [31]. It cannot be excluded that this fraction encompasses 'immature', incorrectly compartmentalized, and unassembled polypeptides as well as whole nonfunctioning complexes. Probably the same viewpoint can be applied to the rapid oscillations in the content of the total cell protein as well as of some individual proteins, including the mitochondrial cytochrome *c* oxidase and ATPase complexes, that were observed by Lloyd and colleagues [32,33] in synchronous cultures of *Acanthamoeba castellanii*. In this case within a 1 h cycle the intracellular level of the enzymes first increased twice and then fell by half.

When considering protein catabolism, the usual topics of discussion are variants of endocytosis or ATP-dependent proteolysis taking place in the cytosol with the participation of a ubiquitin-mediated proteolytic system (see references in [34]). At the same time, it has been mentioned above that control over the assembly of the oligoenzyme complexes of the inner mitochondrial membrane involves endogenous mitochondrial proteinases. This prompts the idea that an important role in the control of the topogenesis of membrane proteins may be played by specific proteinases localized in various subcellular compartments: endoplasmic reticulum [35,36], Golgi complex [37], plasma membrane [38] and chloroplasts [39,40].

Thus, polypeptides constituting membrane multisubunit complexes undergo complicated and prolonged conversions on their way from the site of synthesis to the place of destination in the cell. As this takes place, a large proportion of polypeptides is degraded. Only those are protected from elimination that (i) bind with their partners, (ii) go to the right compartments and (iii) are integrated into functioning complexes. In other words, the regular structure of complexes and supercomplexes and the rigorous compartmentation of separate polypeptides and whole complexes are in no small measure the result of efficient 'supervision' over the assembly, a prominent role in which is played by proteinases.

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