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Biochemistry and Function of Hypusine Formation on Eukaryotic Initiation Factor 5A

The papers assembled in this issue of 'Biological Signals' deal with eukaryotic initiation factor 5A (eIF-5A), which exists in both eukaryotic cells and archaea, but not in bacteria. This protein is the only cellular protein known to contain hypusine. Nature has committed two enzymes, deoxyhypusine synthase and deoxyhypusine hydroxylase, to specifically modify a single lysine residue of eIF-5A into hypusine, as illustrated in figure 1. This is no small commitment from Nature. Indeed, all the literature evidence indicates that eIF-5A, with its hypusine modification, is indispensable for cell survival and proliferation. As noted by Cohen in this issue, the discovery of the essentiality of hypusine in eukaryotic cells has demonstrated a unique and essential role for spermidine in the life of such cells, a fact that is not yet found in texts of biochemistry and cell biology. The highly conserved nature of the eIF-5A sequence from *Methanococcus jannaschii* to human (fig. 2), particularly near the hypusination site, futher confirms the importance of hypusine formation. What remains mysterious is that we do not know the cellular function of eIF-5A and the functional significance of hypusine modification. It is anticipated that, with most of the molecular tools in hand, the cellular function of hypusine and eIF-5A will unfold in time.

Hypusine formation is one of the most specific polyamine-dependent biochemical reactions. It is quite possible that some of the important physiological functions of polyamines are mediated through hypusination of eIF-5A. With that in mind, Cohen provides a succinct review of how the hypusine story began over a quarter of a century ago, and how, in the context of polyamine research, the field has evolved since the early 80s.

Research progress related to hypusine and eIF-5A has been reviewed by Park et al. [1, 2] in 1993. Since then, deoxyhypusine synthase has been purified [3–5], genetic manipulation of either the eIF-5A or deoxyhypusine synthase gene has become possible in yeast and other organisms [6, 7], and a potent specific inhibitor for deoxyhypusine synthase has become available [8]. In this issue, Park et al. review these and other new developments. They also summarize some of their mechanistic studies on the action of deoxyhypusine synthase and functional analysis of this enzyme in the yeast system. Their work provides definitive evidence that hypusine formation correlates with cell proliferation.

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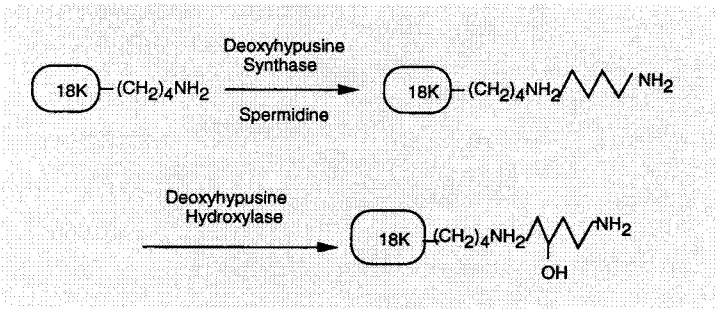

Fig. 1. Schematic diagram illustrating hypusine formation on eIF-5A precursor (18 kD in yeast and mammalian cells, 21 kD in *Neurospora carassa*).

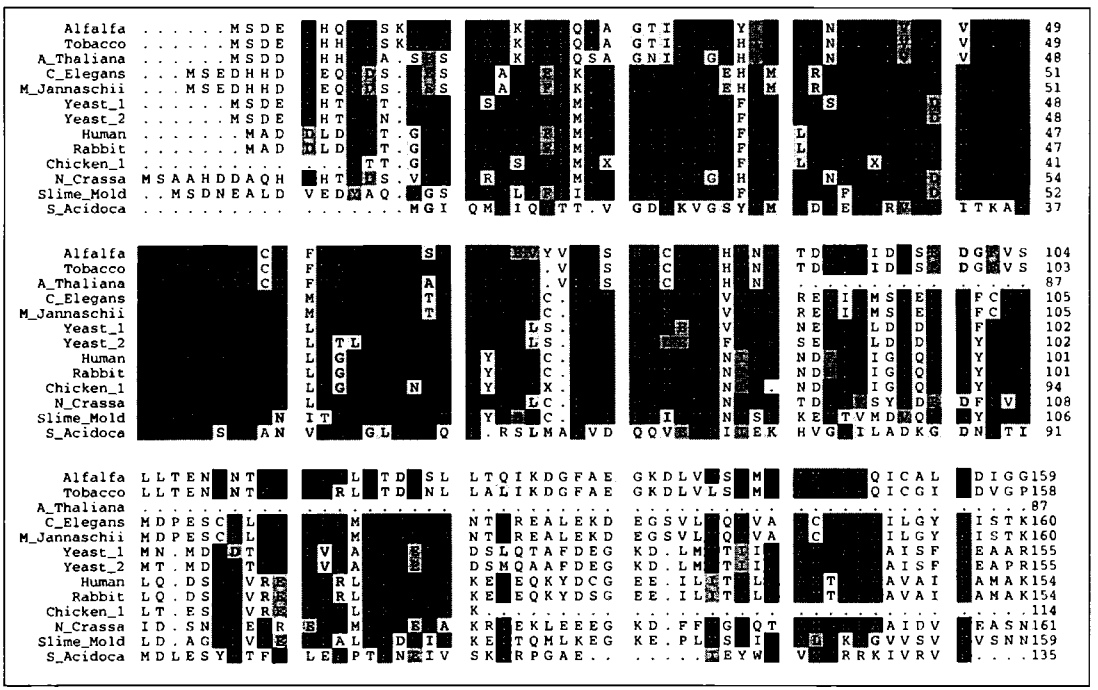

Fig. 2. Alignment of eIF-5A amino acid sequences from 12 different species. Identical amino acid residues are indicated with black solid bocks. Similar amino acids are shaded. The alignment is adjusted for the best match. There are two eIF-5A cDNA for chick and for tobacco, but only one for each species is shown here. Except chicken, spaces introduced for alignment are indicated by a period.

One unexpected finding from Hauber's laboratory in 1993 has linked eIF-5A to the action of the HIV-1 Rev protein. The finding is significant not only because of its implication for AIDS therapy, but also because it suggests a new avenue to probe the action of eIF-5A. A series of papers has since come out of the same group, demonstrating the involvement of eIF-5A in Rev action both in vitro and in vivo. Bevec and Hauber review these

studies and discuss some of their most recent work using the yeast two-hybrid system to search for the cellular targets of eIF-5A.

Using a different experimental approach, work from Shida's laboratory suggests that eIF-5A may interact with the HTLV-1 Rex protein [10]. Their study has substantiated the notion that eIF-5A may be involved in the action of these retroviral proteins as Rex and Rev are functionally interchangeable. Kiyokawa et al. have now extended this finding and demonstrate the presence of at least two distinct pathways for pre-mRNA processing. They suggest that the eIF-5A protein is involved in one of the pathways.

In contrast to these studies, Shi et al. [11] have previously shown that eIF-5A is predominantly localized in the cytoplasm. In this issue, they present additional data which suggest that eIF-5A does not shuttle between the nucleus and cytoplasm in the way Rev does. This is intriguing since colocalization of eIF-5A and Rev would be expected if eIF-5A was the cellular partner of Rev. Clearly, more work is needed to sort out these discrepancies. Thus, although the findings with Rev and Rex are exciting, the overall picture may be complicated. Currently, at least four or five different cellular proteins, including eIF-5A, have been reported in the literature to be the Rev-binding protein.

The essentiality of hypusine in cell growth also points toward its potential role in cell death. Tome and Gerner summarize previous studies on the role of polyamines in cell growth and cell death. They argue that suppression of eIF-5A formation is the cause for apoptosis in a hepatoma variant cell line treated with either diaminoheptane or DFMO withdrawal. Again, if eIF-5A serves as a critical member along the mitogenic signaling pathway, its involvement in apoptosis would not be a surprise. However, detailed analysis of the downstream events from eIF-5A suppression to apoptosis remains to be performed.

Three groups have reported the purification of deoxyhypusine synthase in 1995 [3–5]. The enzyme is a homotetramer, and the amino acid sequence of the enzyme appears to be highly conserved, particularly at the C-terminal half (fig. 3). In this issue, Abid et al. present data on the mutational analysis of yeast deoxyhypusine synthase. They report that repression of deoxyhypusine synthase gene expression results in eIF-5A depletion. It may be interesting to note that, when human eIF-5A was used as the bait in yeast two-hybrid analysis, one of the most consistent true-positive clones that we have identified is deoxyhypusine synthase [Zhu, L. et al., unpubl. results].

Liu et al. discuss their studies on the interaction of eIF-5A with HIV-1 (Rev response element RRE) RNA and U6 snRNA. They found that the deoxyhypusine- or hypusine-modified eIF-5A, but not the eIF-5A precursor, exhibits RNA binding activity. They propose that eIF-5A may be a bimodular protein, capable of both RNA and protein binding. Since the interaction of eIF-5A with RRE or U6 RNA depends on the presence of deoxyhypusine or hypusine modification, RNA binding, as measured by gel mobility shift assay, may serve as an in vitro functional assay for deoxyhypusine or hypusine formation.

Clearly, studies of hypusine in biological systems are just beginning. For example, hypusine in plant is an area largely unexplored at this moment. Regulation of the metabolism of eIF-5A has not been rigorously studied in any system yet. The biochemistry and function of hypusine and eIF-5A in archaebacteria has not been explored either. At the physiological and clinical levels, we wonder what the functional role of free hypusine and other hypusine derivatives in tissue and body fluid

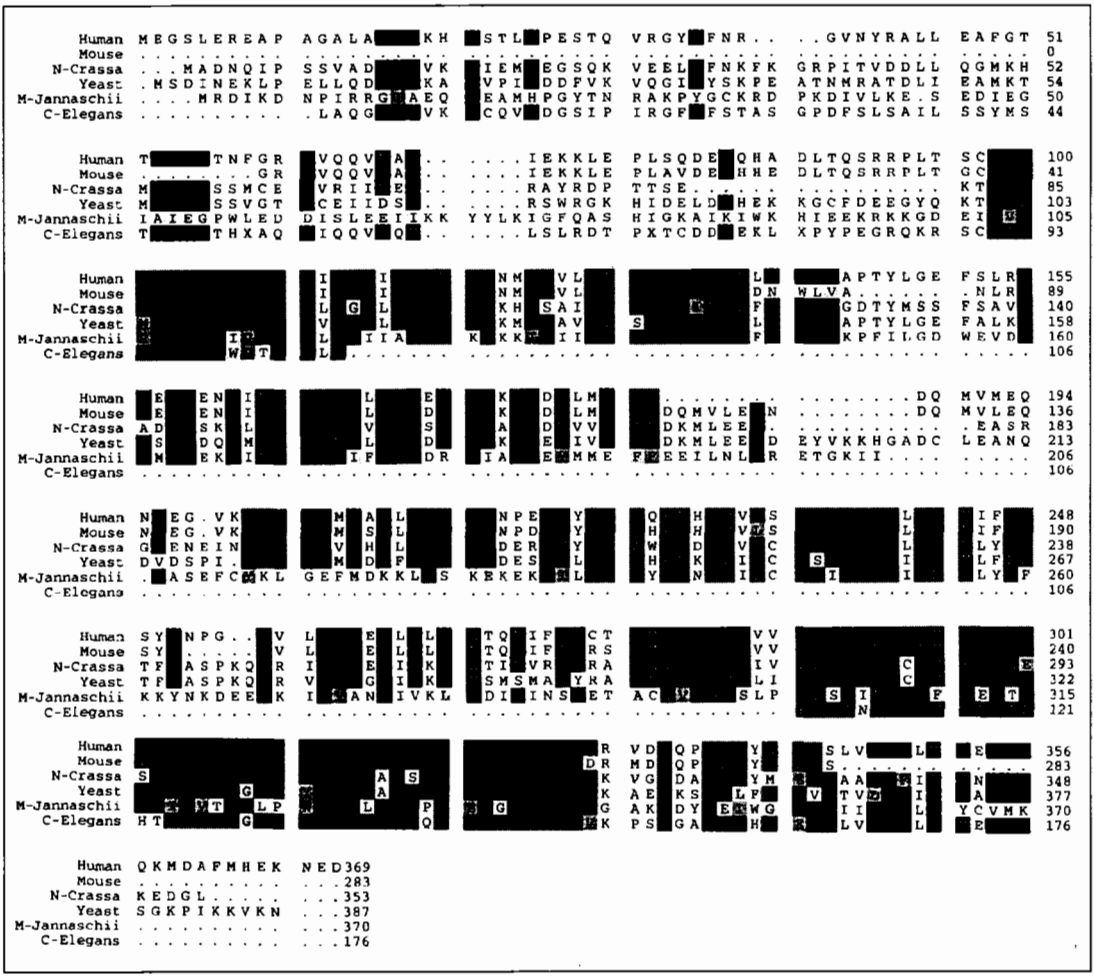

Fig. 3. Alignment of deoxyhypusine synthase amino acid sequences from 6 different species. Identical amino acid residues are shown with solid black blocks and conservative replacement is shaded.

is. For example, we do not know why a high level of hypusine excretion occurs in patients with familial hyperlysinemia [12]. Nor do we know whether GABA-hypusine discovered in the brain [13] functions as a neurotransmitter. Except for deoxyhypusine hydroxylase, which has yet to be purified, the molecular tools for studying the biochemistry and function of hypusine formation are largely in place. Meanwhile, the emphasis of hypusine research is shifting from biochemistry to function. It remains to be seen whether the Rev-RRE system can serve as a useful model for probing the cellular function of eIF-5A. If indeed eIF-5A is a bimodular protein, it will be pertinent to identify the target RNA and proteins of eIF-5A in order to understand its function.

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