Endo- β -D-glucuronidase (heparanase) activity of heat-shock protein/tumour rejection antigen gp96

Graham (1994) reports that the N-terminal ends of gp96 and mouse melanoma heparanase are identical and the two molecules are similar. This identity is indeed provocative, but has to be approached with caution. Sequence data reported by Jin et al. (1990) indicate that the N-terminus detected by Edman degradation is the dominant unblocked N-terminus in that preparation; they do not prove that this sequence indeed resides on the same polypeptide chain which has the heparanase activity. The proof may be arrived at by a demonstration that antibodies against the synthetic N-terminal peptide can neutralize heparanase activity in a specific manner. This has not been shown. We recently reported that gp96 is an ATPase, and this claim was made only after our data met the standard of proof indicated above (Li and Srivastava, 1993). However, it should be noted that, even if antibodies to the synthetic peptides can neutralize enzyme activity, the formal possibility remains that the enzymic activity may be tightly associated with gp96 and the antibody binding may be inhibitory because of steric hindrance.

The reasoning that a number of independent chromatographic steps were used to purify heparanase, and hence it cannot contain a contaminant, is fraught with risks, particularly when the possible 'contaminant' is a member of the chaperone family of proteins. After all, it is the function of chaperones to bind to other molecules. It may be instructive to recall here that gp96 (in a previous incarnation, designated hsp108) was once thought to be a progesterone receptor because it co-purified with the relevant activity through a number of independent chromatographic steps. It became clear later that it was only associated with the receptor (Kulomaa et al., 1986; Sargan et al., 1986).

While remaining cautious about the interpretation of identity between the N-termini of gp96 and mouse melanoma heparanase, two additional similarities between the two molecules may be noted. Although more than 95% of the total gp96 in a given cell is located in the endoplasmic reticulum, a significant proportion of the total gp96 also resides on the cell surface in tumour cells, but not in untransformed cells (A. Altmeyer, R. G. Maki, A. M. Feldweg, V. Protopopov, S. Masur and P. K. Srivastava, unpublished work). This distribution mirrors the activity of the mouse melanoma heparanase (Jin et al., 1990) and is consistent with the proposed role of heparanases in tumour metastasis (Nakajima et al., 1988). The high degree of similarity between the mouse and human tumour heparanase activities (Nakajima et al., 1988) is also reminiscent of the close homology between the mouse and human gp96 genes (Maki et al., 1990; Srivastava and Maki, 1991). However, the gp90 molecule of human melanomas referred to by Graham (1994) is unrelated to gp96, and is a melanotransferrin (Real et al., 1988; Furukawa et al., 1989).

Should it turn out that gp96 is indeed a heparanase, further questions arise. The gp96 belongs to a family of closely related molecules, some of which are encoded by related but different genes. Although we showed a single gp96 gene in mice (Srivastava et al., 1987, 1988) and suggested that only a single coding gene exists in humans (Maki et al., 1993), it is now clear that there is at least one additional gp96 coding gene in humans (P. K. Srivastava, unpublished work) and three to four distinct but serologically related gp96 species in mice (Feldweg and Srivastava, 1993; A. M. Feldweg and P. K. Srivastava, unpublished work). Whether the heparanase activity resides in one or all members of the family will have to be resolved.

In any event, gp96 appears to be an unusually fine example of

evolutionary gene sharing by different functions. Thus gp96 molecules (or genes) (i) possess heat-shock elements in their 5' flanking region and in the first intron (Maki et al., 1990), (ii) bind a diverse array of peptides (Li and Srivastava, 1993; Z. Li and P. K. Srivastava, unpublished work), (iii) chaperone MHC class I (Z. Li and P. K. Srivastava, unpublished work), class II (Schaiff et al., 1993) and immunoglobulin molecules (Melnick et al., 1993), (iv) bind ATP (Li and Srivastava, 1993), (v) possess a Mg^{2+} -dependent ATPase activity (Li and Srivastava, 1993), (vi) contain an interferon-responsive element in the 5' flanking region and are up-regulated in interferon-treated cells (S. L. Anderson, T. Shen, J. Lou, L. Xing, N. E. Blachere, P. K. Srivastava and B. Y. Rubin, unpublished work), (vii) possibly possess an endopeptidase activity (Srivastava et al., 1986; P. K. Srivastava, unpublished work), (viii) have been suggested to contain an MHC-like peptide-binding cleft (Srivastava and Heike, 1991), and (ix) now, possibly possess a heparanase activity! It is safe to reason that, similarly to the situation with crystallins (Piatigorsky and Wistow, 1989), all these properties are not required for each physiological function of gp96. The extent of sharing (or protein recruitment) observed here is all the more remarkable because it has occurred in a relatively short phylogenetic duration: although cytosolic hsp90 genes are present across the entire phylogenetic tree, the endoplasmic-reticulum member of the family, i.e. gp96, appears only in vertebrates among the species tested (P. K. Srivastava, unpublished work). We have suggested that gp96 genes diverged from their hsp90 progenitors in order to cater to an immunological function in mammals and perhaps co-evolved with the MHC (Srivastava and Heike, 1991; Srivastava 1993; Srivastava et al., 1994). Obviously, discovery of newer functions of this remarkable molecule should continue to provide a fertile ground for further evolutionary contemplation.

Pramod K. SRIVASTAVA

Department of Biological Sciences, Larkin Hall, Fordham University, Bronx, NY 10458, U.S.A.

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