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EDITORIAL

FORWARD TO THE PAST! OR, BACK TO PROTEINS

Open any current issue of a journal of endocrinology—molecular, cellular, physiological or comparative—and you are sure to find several papers whose principal findings are based on Northern blots, RNase protection or *in situs*. The major conclusion drawn is usually the same, namely that such and such a hormone (or anti-hormone or drug) regulates a particular gene thought to be important for the action of a given hormonal signal. A large proportion of such studies ignore the protein product of the gene in question. This situation is in contrast to that 25 years ago, when much of biochemical endocrinology was focused on the structural and functional characteristics of proteins important for hormone action. There has, therefore, taken place a wide swing of the pendulum within a generation from protein to RNA.

Undoubtedly, much has been learnt from the measurement of steady-state levels of a given mRNA thought to be under hormonal control. But it is of some interest to consider the reasons for this over-emphasis on measurement of accumulation or disappearance of a given transcript (to be distinguished from the process of transcription itself). First, because it is relatively easy to measure small amounts of mRNAs, in part explained by the continuing technical advances in molecular biology which have spawned a wide variety of easy-to-follow laboratory manuals and simple kits for cloning, sequencing and quantifying RNA. Second, it is commonly assumed (but rarely verified) that the detection of a message and the determination of its concentration reflect the presence, amount or activity of the protein it encodes. Third, determining mRNA levels alone in response to hormonal manipulations may be justified in some cases to explain the response of a target tissue to the hormone. Finally, often it is simply a case of the relatively greater difficulty of identifying, locating and measuring the corresponding proteins present in small amounts in the target tissue. This is especially true for receptors or other proteins which cannot be identified by a distinctive catalytic function or those that are expressed as multiple protein isoforms of a closely related gene family.

Is it necessary to re-direct the current emphasis on mRNA? An increasing number of recent investigations point to the importance of redressing the present situation to that of a more balanced assessment of both RNA and protein. The following few examples should suffice to illustrate this point.

First, consider the question of multiple isoforms. Several members of the steroid/thyroid hormone/retinoid receptor family are expressed as closely related isoforms, as is well known for thyroid hormone (TRs), retinoid (RARs and RXRs) and peroxisome proliferator activator (PPAR) receptors. These may be expressed differentially during development or in a tissue-specific manner to give rise to variable hormonal responses. A Northern blot or RNase protection assay, carried out without an isoform-selective probe, would not be of much use in this respect. Second, one cannot always assume that the location and amount of a given protein is a reflection of those of its mRNA. For example, based on its mRNA distribution, the thyroid hormone receptor isoform $\beta 2$ was considered to be exclusively expressed in the pituitary where it would serve to negatively regulate the TSH gene expression when bound to its ligand. However, a recent study, based on specific immunocytochemical determination of TR$\beta 2$ protein, has revealed that it is expressed in several other cerebral and extra-cerebral tissues, in some cases more extensively than in the pituitary, the same tissues in which TR$\beta 2$ mRNA was present below the limits of its detection. Next, consider the clinically important example of nuclear receptor mutations. Mutations, linked to loss of hormone responsiveness, due to a few or a single amino acid substitution or deletion or frame-shift have been well-documented for human thyroid hormone, androgen and estrogen receptors. These mutant receptors which,

in the absence of sequencing, are indistinguishable from the wild-type receptors by the usual mRNA analyses, not only fail to bind their respective ligands but act in a dominant negative fashion to functionally suppress the normal receptor. Finally, Northern mRNA analysis alone is irrelevant if the receptor or other hormonally regulated protein is dependent on heterodimerization with other members of the nuclear receptor family or general transcription factors. TR, RAR, VDR, PPAR are all thought to function as heterodimers with RXR. It is obvious that a knowledge of protein–protein interaction, and hence working with proteins, is important for the biological activity of the hormonal or non-hormonal ligand.

Clearly then, the above examples emphasize the need for a detailed understanding of protein products to explain hormone action. Indeed, as already mentioned, hormone action was largely studied at the protein level before the advent of molecular biology and the impact of recombinant DNA technology. We have now reached a stage when a balanced and integrated approach to the RNA–protein question is increasingly needed. The writer of this editorial himself was one of those who, a generation ago, was urging researchers to move away from protein to nucleic acids. It is therefore only appropriate for the same person to make a plea for reversing this trend to "back to proteins" for the next phase of furthering our understanding of hormone action.

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