Talking Point

The return of pancreatic ribonucleases

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A decade after losing favor as an 'uninteresting' digestive enzyme, pancreatic ribonuclease has been found to be homologous to a series of extracellular proteins that may influence tumor cell growth, neurological development and biological differentiation. One surprising outcome of these discoveries has been the confirmation of the hypothesis that extracellular *'communicator RNA' is a messenger important in cell growth and differentiation. The only question is: why wasn't this recognized earlier?*

Bovine pancreatic ribonuclease (RNase) was an enzyme central to biochemical research in the 1960s. Anfinsen used RNase as a model for much of his Nobel prize-winning work on protein folding[1]. Saunders chose RNase as the first enzyme to be studied by NMR[2,3]. With the sequences of over 50 homologues known, RNase was an early paradigm of molecular evolution[4].

Biochemical fashions, however, come and go. As with other digestive enzymes, RNase fell from favor in the 1970s. Technological advances made it possible to isolate and study enzymes central to metabolism, making digestive enzymes, initially attractive for study because of their stability and availability in large quantities, apparently less 'relevant'. This sentiment was reinforced in the 1970s when research with transparent 'medical significance' came to be in demand. While *intra*cellular RNases (reviewed recently in *TIBS* by Murray Deutscher[5]) remained interesting, these proteins are generally much larger than (and generally not homologous to) the simpler digestive enzyme.

However, sometimes fashions return. For example, no sooner had granting agencies and others in tune with the excitement of the moment decided that digestive proteases were 'uninteresting', roles for proteases in key regulatory processes were discovered. Today, we know that proteases play central roles in genetic regulation, viral replication and the induction and growth of tumors[6].

RNases seem destined to undergo an analogous revival, and the prospect will warm the heart of anyone with a fondness for the underdog in the face of conventional opinion. *Extra*cellular RNases appear now to play important roles as factors controlling growth and development in higher organisms. Further, *extra*cellular RNA molecules, the logical substrates for an extracellular RNase, have just been rediscovered, and roles for these molecules in cellular growth and development have just been confirmed[7–11].

A biological role for extracellular RNA and RNases might have been suspected a decade ago, especially by those who were in tune with issues in molecular evolution. In 1969, Barnard remarked, in a paper in *Nature*[12], that digestive RNases were abundant only in specialized groups of animals, particularly in ruminants and certain other herbivores. As ruminant digestion is probably less than 60 million years old, this observation suggested that a digestive role for RNase is itself a recent evolutionary innovation, arising from secreted RNases that played other roles. It was appropriate then (and remains appropriate now) to wonder: did extracellular RNases perform another role *before* they became useful in ruminant digestion? If so, might this role still be performed by some modern RNases?

Non-digestive extracellular RNases are certainly known in the serum of mammals and elsewhere[4,13,14]. A RNase from seminal fluid has proven to be especially interesting[15]. Seminal RNase is a dimer and, unlike monomeric pancreatic RNase, has substantial activity against double-stranded nucleic acids[16,17]. Further, seminal RNase has long been known to be a potent inhibitor of tumor cell growth, both *in vitro* and *in vivo*[7,18–20].

Of course, it is possible that seminal RNase must be transported into a cell for it to exert its anti-tumor activity. However, it is no less plausible for the site of action to be extracellular,

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provided that RNA also exists extracellularly, and can there serve as the substrate for the RNase. From this perspective, the biological activity of seminal RNase implies the existence of a new sort of RNA molecule, one serving a biological function outside the cell, awaiting discovery.

Many early experiments hinted that such extracellular RNA molecules might well exist. For example, in the mid-1970s, Kolodny showed that macromolecular RNA was transferred from cell to cell in tissue culture[21]. Even earlier, Folkman reported that tumor angiogenic factor (TAF), a substance presumably secreted by solid tumors to attract blood vessels essential for nourishment and growth, lost its biological activity when treated with RNase[22]. If extracellular RNA were necessary for tumor angiogenesis, a working hypothesis explaining the antitumor activity of extracellular RNases would be plausible. Such a hypothesis might have stimulated experimental work to detect extracellular RNA and RNases involved in growth and development.

However, these data were scattered and, in some cases, buried in papers whose main point was something else. The results were therefore easily forgotten as unexplainable esoterica often found in biomedical research. Further, anyone who might have found these data significant and sought to examine the hypothesis experimentally had a still more difficult obstacle of convincing funding agencies to abandon their opinion that RNase was an uninteresting digestive enzyme, and that RNA belonged inside cells and not outside.

Fortunately, experimental evidence overturned this view. In 1985, Vallee and his group (supported by private grants from the Monsanto corporation) isolated another tumor angiogenesis factor, 'angiogenin'. The angiogenin sequence was found to be 35% identical to the sequence of digestive RNases[23,24]. In some ways, human angiogenin resembles turtle RNase more than human RNase[25], suggesting a divergence of these types of proteins early in the evolution of higher organisms (and well before the origin of digestive RNase in ruminants).

A year later, two more RNase homologues with interesting biological properties were discovered in eosinophils; these RNases caused the degeneration of nervous tissue[26]. A role for extracellular RNases in biological development (albeit abnormal development) was thus better established[27].

Still more recently, Wissler and his colleagues in Germany found that 'angiotropin', an extracellular factor that stimulates the growth of blood vessels, is a ribonucleoprotein containing an RNA component approximately 75 bases in length[9–11]. Wissler's angiotropin may be the same growth factor as Folkman's tumor angiogenic factor; the present characterization of the two substances does not rule out this possibility. However, Wissler's exciting work indicates that extracellular RNA exists, and strongly suggests a role for extracellular RNA in normal cell growth and development.

Extracellular RNases, extracellular RNA molecules, and extracellular RNase inhibitors form the triad necessary for a functioning regulatory system. At the very least, more examples of these classes of biological regulators are likely to be found as regulators of interesting biological processes.

Further, experimental data establishing the structural basis for the remarkably diverse biological activities of different RNase homologues should emerge in the next few years. The first hybrids of pancreatic RNase and angiogenin have just been prepared by recombinant DNA techniques (Ref. 29 and Allemann, R. K. [1989] Dissertation, ETH, Zurich). One of the more interesting hybrids has nine amino acids from angiogenin replacing the corresponding region in pancreatic RNase (Allemann, R. K., op. cit.). The result is a protein sequence 92% identical to that of digestive RNase. However, the catalytic activity against *small* RNA substrates characteristic of the digestive enzyme is diminished in the hybrid by over 98%, and the catalytic activity against *large* RNA substrates (apparently characteristic of angiogenins) is increased (Allemann, R. K., op. cit.).

Results like these should make RNases once again the focus of biological excitement. The train of speculation and hypothesis outlined above illustrates how ideas from molecular evolution, together with individual pieces of reliable data, can stimulate scientists in far-removed fields. For the biochemist and funding agency alike, RNase provides yet another example of the resilience of basic research. Further, the resurrection of RNase as a research topic should provide new hope for all of us who nurture a fondness for a biochemical problem that has fallen from favor.

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