'Zip codes' direct intracellular protein tyrosine phosphatases to the correct cellular 'address'

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The transmembrane and intracellular protein tyrosine phosphatases (PTPs) play an essential role as signal transduction proteins involved in various cellular processes including division, proliferation and differentiation. As such, their activity must be strictly regulated to avoid nonspecific tyrosine dephosphorylation of cellular proteins. The intracellular PTPs possess a diversity of protein sequences outside the catalytic domain that appear to serve as 'zip codes' specifically 'addressing' these proteins to defined subcellular compartments. These localization strategies are proposed to function as a regulatory mechanism, defining the substrate specificity and function of the intracellular PTPs.

THE PROTEIN TYROSINE phosphatases (PTPs) are a growing family of enzymes that function, in concert with protein tyrosine kinases, to modulate the tyrosine phosphorylation of cellular proteins (for comprehensive reviews, see Refs 1 and 2). Of the 30 or so PTPs characterized to date, approximately a third are transmembrane, receptor-like molecules, while the majority are cytoplasmic proteins. With such a large number of potentially redundant enzymes located intracellularly, the activity of the intracellular PTPs must be highly regulated to prevent spurious, nonspecific dephosphorylation of proteins. Regulation of the activity of such cellular enzymes can be accomplished in various ways: (1) by modulating the steady-state levels of the enzyme; (2) by substrate specificity; (3) by post-translational modification (i.e. phosphorylation, proteolytic cleavage, etc.); and (4) by interaction with inhibitor/activator molecules. We are just beginning to understand how these regulatory mechanisms might function to modulate PTP activity. In particular, controlling substrate specificity by restricting substrate availability could be an important means of regulating the intracellular PTPs. Sequence analyses of the PTPs have revealed an exquisite diversity of protein sequences outside the highly conserved catalytic domain. It has been suggested that these noncatalytic sequences serve as 'zip codes' to 'address' the PTPs to specific compartments within the cell, thereby defining and restricting their substrate specificity. Here we will discuss several examples of intracellular PTPs that could be subjected to this mode of regulation.

The PTP family

The PTPs are multidomain proteins whose structural features separate the family into two main classes based on their transmembrane or intracellular location (Fig. 1). All PTPs, whether transmembrane or cytoplasmic, possess at least one 230 amino acid catalytic domain containing a highly conserved active-site region with the consensus motif [I/V]HCXAGXXR[S/T]G (where X is any amino acid). This region is the signature sequence of most PTPs. Site-directed mutagenesis and trapping experiments have shown that the cysteinyl residue within this motif is essential for phosphatase activity and forms a thiol–phosphate intermediate during catalytic turnover[1]. Interestingly, this catalytic domain bears no resemblance to that of the serine/threonine phosphatases or of the alkaline or acid phosphatases.

Transmembrane PTPs possess an extracellular domain, a single transmembrane domain and normally two catalytic domains followed by a short carboxy-terminal segment. At present, the human PTPβ and the *Drosophila* DPTP10D are the only transmembrane proteins that have a single catalytic domain. Unlike the cytoplasmic tail, the extracellular domains of these proteins are highly divergent, with small glycosylated segments (human PTPα and ε), tandem repeats of immunoglobulin-like and fibronectin type III domains similar to N-CAM molecules [leucocyte common antigen related molecule (LAR), PTPμ], or alternately spliced lengths of sequence containing N- and O-linked carbohydrates (CD45). These extracellular features suggest that the activity of these PTPs might be modulated by ligands. Recently, it was shown that the homophilic binding of PTPμ can mediate cell–cell aggregation[3,4]. This suggests that the extracellular domain of this PTP can serve as its own ligand and could potentially function to regulate cell–cell interactions.

The distinguishing feature of the intracellular PTPs is the diversity of domains flanking a single catalytic domain (Fig. 1). One of the principle functions of these flanking sequences, or zip codes, appears to be the targeting of the enzyme to specific intracellular locations. These include: (1) membrane-association domains, (2) nuclear-localization domains, (3) Src homology 2 (SH2) domains and (4) cytoskeletal-association domains. A schematic showing these intracellular PTPs residing at their proven or hypothesized subcellular locations within a eukaryotic cell is shown in Fig. 2. We will discuss each of the PTPs shown in the following sections.

Membrane and nuclear targeting sequences for PTPs

A number of intracellular PTPs possess unique sequences downstream of the catalytic domain, which might serve to regulate the targeting of the protein to a subcellular locale and, in some cases, also modulate phosphatase activity. Purification of PTP1B from the human placenta yielded both soluble and particulate forms of the enzyme,

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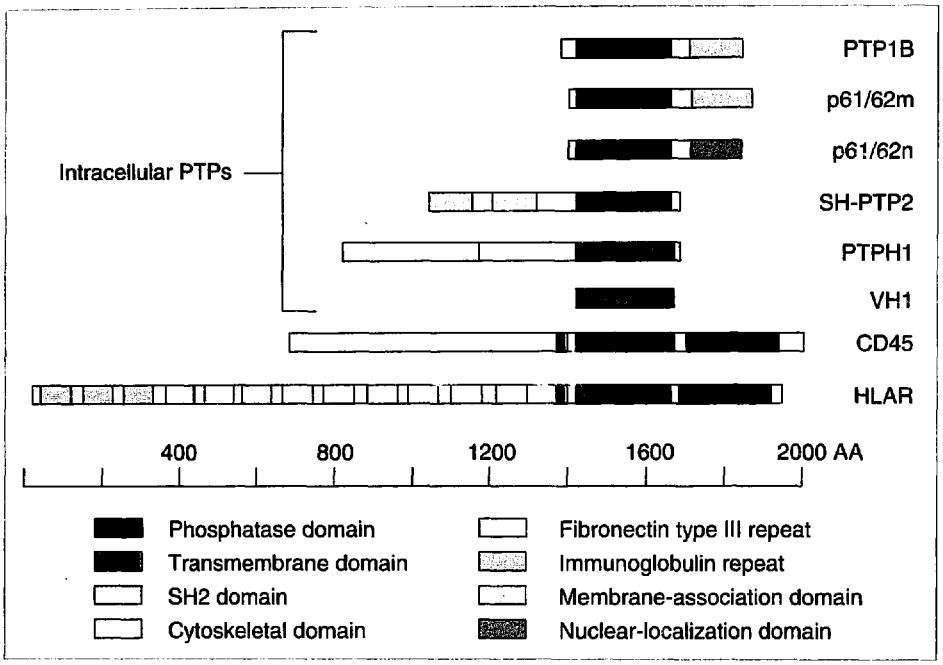

Figure 1

Structural features of representative transmembrane and intracellular PTPs. Each PTP possesses one or two highly conserved catalytic domains. The intracellular PTPs pictured have noncatalytic sequences including membrane association domains (PTP1B), Src homology 2 (SH2) domains (SH-PTP2) and cytoskeleton-association domains (PTPH1). The transmembrane PTPs pictured have a single transmembrane domain and extracellular domains with sequences containing amino-terminal O-linked carbohydrates (CD45) and tandem repeats of immunoglobulin-like and fibronectin type III domains (HLAR). A line scale indicates the approximate number of amino acids in each protein.

and amino acid sequencing revealed the soluble PTP1B to be a 321 amino acid protein[5]. Subsequent cloning of the corresponding cDNA predicted the existence of an additional 114 amino acids at the carboxyl terminus[6]. The rat homolog of PTP1B, known as PTP1, also possesses a carboxy-terminal extension of 111 amino acids and, when expressed in *Escherichia coli*, consistently yields an active protein associated with the particulate fraction[7]. The phosphatase lacking the carboxy-terminal sequence is soluble, suggesting that the deleted sequence is responsible for membrane association. Examination of the extreme carboxy-terminal sequence of these proteins reveals a prominent hydrophobic region of 20 amino acids flanked by charged residues (Fig. 3). Studies employing reporter proteins to determine the importance of this sequence in subcellular targeting have shown that this region is critical and adequate for the cellular localization of full-length PTP1B and PTP1 to the endoplasmic reticulum (ER)[8,9].

Although we can verify the localization of PTP1 and PTP1B to the ER, we can only speculate about the functional reasons for this localization. Compartmentalization to the ER could serve merely as a 'way-station' until a cellular stimulus induces translocation of the enzyme to another compartment where it is active. Recent studies have shown that such a scenario might occur following agonist-induced platelet activation[10]. The carboxyl terminus of PTP1B appears to be cleaved, resulting in translocation of the protein from membranes to the cytosol as well as a twofold increase in PTP catalytic activity. While localized to the ER, these PTPs could also dephosphorylate proteins that reside on, or are closely associated with, the ER (Fig. 2). Tyrosine kinases, such as Ltk[11] and Src-related tyrosine kinases[12], are found along the ER, within focal adhesions [regions of plasma membrane and complexed intracellular proteins associated with the extracellular matrix (ECM)] and adherens junctions (sites of cell–cell attachment). Recently, a new tyrosine kinase, FAK (focal-adhesion kinase), has been described; it also localizes to focal adhesions in fibroblasts and may associate with c-Src and cytoskeletal proteins such as paxillin and talin[13]. PTP1 overexpressed in Src-transformed fibroblasts appears to be localized to focal adhesions as well as to the ER[14]. One of these PTPs could be localized near, or associated with, these ER- and adhesion-specific kinases, and could act to directly regulate kinase activity or that of their substrates.

The human T-cell PTP is an example of an intracellular PTP whose activity appears to be regulated by the presence of its carboxy-terminal sequence. This phosphatase has two carboxy-terminal isoforms: the originally isolated PTP with a long carboxyl terminus rich in hydrophobic amino acids [referred to as T-cell PTP(a) in Fig. 3] and an identical protein possessing a shorter, hydrophilic carboxyl terminus [T-cell PTP(b) in Fig. 3]. Overexpression of the full-length T-cell PTP(a) in baby hamster kidney (BHK) cells results in association of the protein with the particulate fraction[15]. This PTP has no apparent effect on the growth or morphology of the cells and, in fact, shows minimal phosphatase activity towards artificial phosphotyrosine substrates. Limited trypsinization of the extracts releases a truncated, soluble form of T-cell PTP(a) lacking the carboxy-terminal extension but exhibiting fivefold higher enzyme activity. The expression of the truncated T-cell PTP(a) in the BHK cells results in cytokinetic failure, asynchronous nuclear division and suppression of the transformed phenotype. Expression of this PTP in v-Fms-transformed cells also results in a similar attenuation of the neoplastic phenotype[16]. These studies reveal an interesting means of regulating PTP activity: the carboxy-terminal extension might act as a negative regulator of enzyme activity as well as a targeting domain for membrane association.

A novel *Drosophila* PTP was recently isolated that is targeted to two distinct intracellular locations due to alternative splicing[17]. The gene encoding this phosphatase, known as *DPTP61F*, undergoes alternative splicing at the

3'-end of the message, resulting in two RNA transcripts that encode proteins with discrete carboxy-terminal tails. The carboxyl terminus of the longer protein, p61/62m, contains 24 hydrophobic amino acids that serve as a membrane-association signal. The shorter isoform, p61/62n, has a sequence containing 11 hydrophilic amino acids within this region that resembles the nuclear-targeting consensus sequence (Fig. 3). Overexpression of p61/62m in COS-1 cells led to membrane localization of the protein to a reticular network (possibly ER) as well as to mitochondrion-like organelles. By contrast, p61/62n localized solely to the nucleus. Kinetic analysis of these two proteins revealed no difference in catalytic activity or substrate specificity. These results suggest that the activity and substrate specificity of these isoforms is determined by their subcellular localization which, in turn, is determined by the carboxy-terminal zip code. In addition, the isolation of a nuclear PTP is interesting since it could conceivably function in regulating gene transcription by modulating tyrosine-phosphorylated transcription factors[18], or in regulating cell-cycle proteins (Fig. 2).

SH2 domains link PTPs to signaling pathways

The SH2 domain is a conserved sequence motif of approximately 100 amino acids known to have a fundamental role in regulating interactions of cytoplasmic signaling molecules with receptor tyrosine kinases[19]. This domain was originally identified in the v-Src family of non-receptor tyrosine kinases and has since been found in a wide variety of proteins. These include: other non-receptor tyrosine kinases such as Abl; signaling enzymes such as phospholipase Cg and the 85 kDa subunit of phosphoinositide 3-kinase; and the putative oncogenic transcription factor known as Vav. In all of these proteins, the SH2 domain is thought to function as a linker by binding to a phosphotyrosine residue on activated (i.e. autophosphorylated) growth factor receptors, thus bringing together multiple components of the appropriate mitogenic signaling pathway. Several PTPs appear to contain SH2 domains. Two of the SH2-containing PTPs that have been studied in some detail are SH-PTP1, which is expressed predominantly in hemopoietic cells (also referred to as PTP1C, HCP and SHP), and SH-PTP2 (SYP, PTP1D, PTP2C and SH-PTP3), which is a ubiquitously expressed protein. These highly homolo[illegible]s all possess a single catalytic domain with two tandem amino-terminal SH2 domains (Fig. 1). Recent studies have shown that these PTPs interact with other signaling molecules such as activated platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and the insulin receptor substrate 1 (Refs 20–23). In addition, mitogenic stimulation of various cell lines with PDGF, EGF or colony stimulating factor-1 leads to an increase in tyrosine phosphorylation of the PTP protein on an unidentified tyrosine residue.

These studies demonstrate that the SH2 domains enable PTPs to localize to and interact with the phosphotyrosine residues of activated growth factor receptors (Fig. 2). Once associated with the receptor, the PTP might then function to modulate the transduction of the mitogenic signal within the cell. Since it appears that only a small percentage (0.5–1%) of the SH2-containing PTP in the cell translocates to the receptor following stimulation, this targeting probably serves to place the protein near key substrates. The remaining cytoplasmic protein could be an inactive pool or might interact with other phosphorylated signaling proteins downstream in the pathway. In addition to localizing the PTP to substrates at the membrane, the binding of the PTP to the phosphotyrosine residue of the receptor and the subsequent phosphorylation of the PTP might influence enzyme activity. Preliminary *in vitro* experiments have shown that,

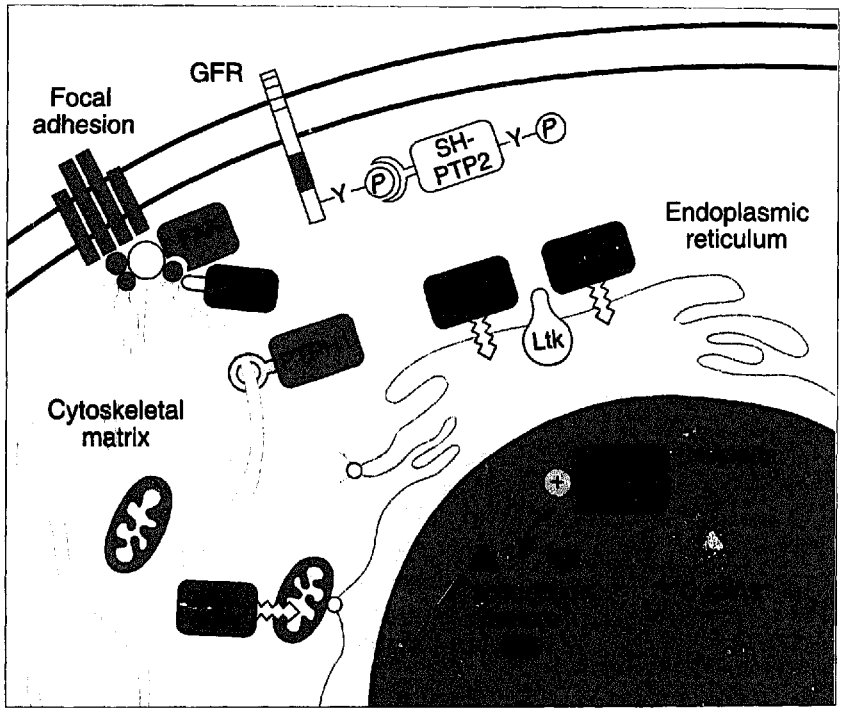

Figure 2

Distribution of intracellular PTPs within the eukaryotic cell. This schematic shows the distribution of the intracellular PTPs at proven or speculated locations within the cell. Each PTP is pictured as a catalytic 'box' with an associated domain that is the zip code for that compartment. At the plasma membrane we might find a PTP (? PTP), such as PTP1, associating with focal adhesions, along with the adhesion-specific tyrosine kinase FAK. In addition, an SH2-containing PTP, like SH-PTP2, would be associated with an activated growth factor receptor (GFR) as a result of translocation from the cytoplasm following an extracellular mitogenic signal. We might find a cytoskeletal PTP, such as PTPH1, interacting with cytoskeletal matrix proteins. The intracellular membrane compartment would be home to PTP1B, which would be lodged in the ER membrane along with the ER tyrosine kinase Ltk. The membrane isoform of the *Drosophila* DPTP61F (p61/62m) would also be here, on the ER and/or the mitochondrial membrane. Within the nucleus, we would find the nuclear isoform of DPTP61F (p61/62n) which might function in regulation of gene transcription or cell division.

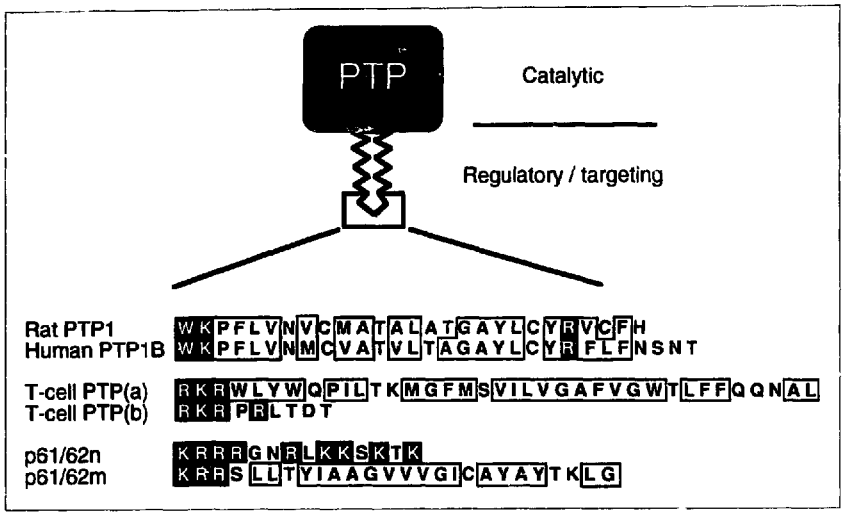

Figure 3

Carboxy-terminal sequences of some PTPs regulate membrane and nuclear localization. These PTPs can be thought to possess two major regions: an amino-terminal catalytic region and a carboxy-terminal targeting/regulatory (T/R) region, as pictured. The most carboxy-terminal amino acid sequence of the T/R region is shown for the human and rat PTP1B homologs, for the human T-cell PTP isoforms and for the *Drosophila* DPTP61F isoforms. Amino acids boxed in black are charged residues; those boxed in white are hydrophobic residues. The PTP1B homologs have a sequence of 20 hydrophobic residues flanked by charged residues, which resembles a membrane-spanning region. The T-cell PTP isoforms exhibit a long stretch of hydrophobic residues [T-cell PTP(a)] or a shorter stretch of charged residues [T-cell PTP(b)]. The *Drosophila* PTP, DPTP61F, also has two isoforms: a longer protein exhibiting a potential membrane-spanning region similar to PTP1B (p61/62m) and a shorter form with a potential nuclear localization consensus sequence (p61/62n).

when a tyrosine-phosphorylated peptide containing the PDGFR site binds to SH-PTP2, the phosphatase activity increases[21]. The same PTP (named PTP1D by Vogel *et al.*[24]), when overexpressed in human embryonic kidney cells along with PDGFRβ, exhibits enhanced catalytic activity following tyrosine phosphorylation. Collectively, these studies strongly suggest a physiological function for these PTPs, but their specificity, if any, for particular signaling pathways remains to be shown. Work with the *Drosophila* gene *corkscrew*, encoding an SH2-containing PTP, has shown that the corkscrew protein appears to function in concert with the c-Raf homolog pothole, to transduce a positive signal from the receptor protein tyrosine kinase torso[25]. The integrity of this pathway is essential for the normal formation of anterior and posterior structures during embryogenesis. The importance of the murine homolog of SH-PTP1 (also called Hcph) in the development and function of the immune system is exemplified by the severe defects in hemopoiesis exhibited by mice homozygous for mutations at the *motheaten* locus on chromosome 6 (Ref. 26). Point mutations in the gene encoding Hcph cause aberrant RNA transcripts resulting in a lack of, or decreased activity of, the PTP. Further studies with this animal model should assist in determining the relevant signaling pathways for one of the SH2-containing PTPs.

Cytoskeletal PTPs: interior designers of the cell?

A unique amino-terminal targeting domain has been identified in several intracellular PTPs which has sequence similarity to known cytoskeleton-associated proteins. These domains within PTPMeg1 and PTPH1 have up to 45% homology with amino-terminal domains in the erythrocyte proteins band 4.1, ezrin and talin, which are all members of the band 4.1 superfamily of cytoskeletal proteins. In general, members of this superfamily are responsible for the association of actin filaments with a complex of proteins at the plasma membrane. These protein associations are critical in the control of cell shape and motility; in defining specialized membrane regions such as synaptic densities; and in stabilizing cell–cell and cell–matrix interactions[27]. Band 4.1 binds to the erythrocyte transmembrane proteins band 3, glycophorin C and spectrin, enhancing spectrin–actin affinity. The interaction with band 3 is mediated by amino-terminal sequences, suggesting that similar interactions, involving the amino-terminal domains of the cytoskeletal PTPs with members of this family of cytoskeletal proteins, might occur in the cell. In addition to PTPMeg1 and PTPH1, a related PTP, BA14, has been isolated from bovine adrenal tissue and possesses a similar cytoskeletal-association domain along with five GLGF motifs[28]. GLGF motifs have been identified in proteins that reside in specialized junctions, such as tight junctions and postsynaptic densities.

The identification of PTPs with domains related to cytoskeletal proteins adds an exciting facet to the regulation of cytoskeleton–plasma-membrane interactions (Fig. 2). Although the association of these PTPs with such proteins has yet to be proved, our present knowledge of the role of tyrosine phosphorylation in the maintenance and modulation of cell–cell and cell–substratum interactions strongly suggests a potential function for PTPs localized to these cellular regions. An example is the sculpting of focal adhesions by the changes in tyrosine phosphorylation of associated proteins[13,29,30]. Focal adhesions consist of transmembrane proteins known as integrins, which link the ECM to an intracellular plaque associated with actin bundles (stress fibers). Attachment of cells to ECM proteins results in tyrosine phosphorylation of multiple intracellular proteins associated with these adhesions including paxillin, tensin, talin, β-integrin and FAK. Inhibition of cellular tyrosine kinases prevents formation of focal adhesions. It is not hard to imagine a cytoskeleton-associated PTP functioning in this integrin-mediated tyrosine phosphorylation, as well as in other cell processes regulated by cytoskeleton–plasma-membrane interactions.

Overview

The structural features of the intracellular PTPs and the experimental evidence concerning location–function relationships of these molecules suggests that the targeting of the PTPs is an important mechanism used to modulate PTP activity in the cell. This means of

regulatory localization probably serves to define substrate specificity and function. There is growing evidence that such localization strategies are used to regulate many cellular enzymes. The serine/threonine kinases and phosphatases often associate with specific cellular proteins, referred to as targeting subunits, which restrict their subcellular localization, defining the time and place of subsequent catalysis. For example, the well-characterized glycogen-associated protein phosphatase-1 (PP1), involved in the regulation of glycogen metabolizing enzymes, exists as a heterodimer complexed with a glycogen-targeting subunit[31]. This additional subunit possesses a glycogen-binding domain, a sarcoplasmic reticulum membrane-association domain and phosphorylation sites that, when modified, can regulate the activity of the complexed PP1. Functionally, this is similar to the intracellular PTPs in which domains serve to confine the enzyme to a specific cellular space. Structurally, these PTPs differ in possessing such domains as a part of the catalytically active protein. At present, it is not known whether or not a similar class of subunit proteins exists that associate with intracellular PTPs, providing additional targeting information and/or regulating catalytic activity. Future research should determine the role of these non-catalytic zip codes in the regulation of PTP activity during various cellular processes.

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Sidney and the sage

Sidney the worm felt very put down
As the object of study by a sage of renown;
Poked and prodded, his innards exposed,
He suffered in silence when with chemicals dosed.
'This invasion of privacy is an outrage,'
Thought Sidney, 'I like not the work of this sage,
For what he proposes surely ill bodes
For me and all other nematodes.'
Said the sage to Sidney, 'My vermiform friend,
I've learned all about you from front to end,
Every cell has its place and provenance too,
I have found where they come from and all that they do.
You are to me like an open book
For with insight and genius I knew how to look,
Secure in conviction that determinism
Encompasses all without doubt or schism.
Now by control of genetic transcription
I can change you according to my prescription.
Such power no man has had before –
God-like, they called it, in days of yore.'
Asked Sidney, 'Am I merely the sum of my genes,
Religion and culture the constructs of memes?[1]
Am I defined without subjective self –
A molecular object plucked from the shelf?
Are collective unconscious and Freudian dreams
Just chemical fluxes in neuronal streams?
If all is the outcome of chance mutation
How then life's meaning is Art's creation?
Despite exegeses and reductionist charts
The whole is still greater than the sum of the parts.
Molecular accounts of life leave no room
In their equations for *cogito ergo sum*,
Yet past, present and future – all, so they say,
Is the dominion of snippets of DNA
Whose enthronement, it is plain to see,
Is the stuff of a new doxology.'
'Methinks', said Sidney, 'this dogma biological
Is a short remove from credo theological.'
But the sage was certain his work would unveil
The secrets of life – the sought holy grail.
Said he, 'I have triumphed, it will be no surprise
To receive the call for the Nobel prize.'
But when hubris will out the great are cast down,
And such was the fate of this sage of renown,
For one day he stumbled and fell on his head,
So Sidney and friends gathered and ate him instead.

1 Dawkins, R. (1989) in *The Selfish Gene*, p. 192, Oxford University Press

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Calling all budding biochemical poets: *TIBS* would welcome your contributions.