Genetic Evidence for the Expression of ATP- and GTP-specific Succinyl-CoA Synthetases in Multicellular Eucaryotes*

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Highly ATP- and GTP-specific isoforms of succinyl-CoA synthetase in pigeon incorporate the same α-subunit, but different β-subunits (Johnson, J. D., Muhonen, W. W., and Lambeth, D. O. (1998) J. Biol. Chem. 273, 27573–27579). The sequences of the mature subunits were determined by methods based on reverse transcription-polymerase chain reaction. The 306-residue mature α-subunit in pigeon shows >88% identity to its homologues in pig and rat. The sequences of the mature ATP- and GTP-specific β-subunits (A-β and G-β, respectively) in pigeon are 54% identical. These sequences were used to identify expressed sequence tags for human and mouse that were highly homologous to G-β and A-β, respectively. The sequences for mature A-β and G-β in mouse and human were completed and verified by polymerase chain reaction. The sequence of A-β in pig was also obtained. The mammalian A-β sequences show >89% identity to each other; the G-β sequences are similarly related. However, pairwise comparisons of the A-β and G-β sequences revealed <50% identity. Alignment with two sequences of the β-subunit in Caenorhabditis elegans suggests that the A-β and G-β genes arose by duplication early in the evolution of multicellular eucaryotes. The expression of A-β is strong in numerous mouse and human tissues, which suggests that ATP-specific succinyl-CoA synthetase also plays an important role in species throughout the animal kingdom.

For over 40 years, the widely accepted model for the Krebs cycle in animals has presumed the participation of GTP-specific succinyl-CoA synthetase (G-SCS; EC 6.2.1.5). An ATP-specific succinyl-CoA synthetase (A-SCS; EC 6.2.1.4) has long been known to occur in plants (3). Although bacterial enzymes including the well characterized Escherichia coli enzyme were first thought to be ATP-specific (4), later studies showed a broader specificity, with nucleotide preference varying widely with species (5, 6). The first evidence for occurrence of A-SCS in the animal kingdom included extensive purification of A-SCS from blowfly (7) and partial purification from pigeon breast muscle (8, 9). Evidence that A-SCS can generally be found in animals has come mainly from enzyme assays of various tissues in a wide range of species (10–12). Studies of the inhibition of SCS by vanadate (13) and labeling of the α-subunit of SCS by γ-labeled ATP and GTP (14) have been interpreted as indicating the presence of A-SCS in some rat tissues. Despite these types of preliminary studies, A-SCS has not been extensively characterized in any species; its occurrence and role in animals remain relatively unexplored; and the genetic relationship between A-SCS and G-SCS is unknown. In the accompanying paper (15), we present data from enzyme purification and characterization that both A-SCS and G-SCS are present in pigeon, although differentially expressed in various tissues. We further show that nucleotide specificity must be determined by the β-subunit since the same α-subunit is incorporated in both enzymes.

All SCSs studied thus far have α- and β-type subunits. The enzyme is dimeric in eucaryotes and Gram-positive bacteria, but tetrameric in Gram-negative bacteria (16). The α-subunit is phosphorylated on a histidinyl residue as a part of the catalytic cycle (17). Recent workers have continued to assume that the α-subunit possesses the determinants of nucleotide specificity (18), a possibility we have shown to be untenable (15). The crystallographic structure of the E. coli enzyme is available (19).

Extensive work on the genes encoding SCS have been carried out on the ATP-preferred enzyme in E. coli (20) and the GTP-specific enzyme in pig (21, 22). These studies, together with the completed genome projects for several procaryotes and yeast, show that the α- and β-subunits are products of separate genes.
Multiple genes are present for the α-subunit of SCS in *Trichomonas vaginalis* (23); however, information is lacking as to the number and nucleotide specificity of isoforms expressed. An alternative splicing mechanism for the α-subunit in pig generates two isoforms of SCS that are GTP-specific (24). Prior to the work described here, the only sequence reported for the β-subunit of SCS in a vertebrate species was for G-SCS from pig (22).

Here, we report the results of obtaining the sequences of the β-subunits of A-SCS and G-SCS in pigeon, human, and mouse; A-β in pig; and the α-subunit in pigeon. We also show that mammalian species express the genes for both forms of SCS in a wide variety of tissues. These results suggest the need to reexamine the occurrence and respective roles of A-SCS and G-SCS in animals and to reassess the regulation and integration of guanine and adenine nucleotide metabolism in the matrix of mitochondria.

**Experimental Procedures**

**Nucleic Acid Preparations for Cloning—** Messenger RNA was isolated from pig tissues and pig heart using the Dynal Dynabeads oligo(dT) direct kit; mRNAs from mouse heart and human adult and fetal liver were obtained from CLONTECH. Pools of total DNA were reverse-transcribed 0.5 μg of mRNA for 1 h at 24 °C using an oligo(dT) anchor primer (CGTCAATGACGATCCGACGCAGTCCATTAG(T17)) and murine leukemia virus reverse transcriptase from Perkin-Elmer. The total cDNA pools were purified using GlassMAX preparatory columns (Life Technologies, Inc.). Oligonucleotides for RT-PCR were obtained from Great American Gene Co. and Integrated DNA Technologies, Inc. Products from PCR were blunt end-cloned into pT7Blue plasmids (Novagen), and both strands of each clone were sequenced by Macromolecular Resources at Colorado State University.

**Conditions for PCR—** The following conditions were used with degenerate oligonucleotide primers: an initial cycle at 94 °C for 60 s, 45 °C for 90 s, and 65 °C for 5 min; 28 cycles at 94 °C for 60 s, 52 °C for 90 s, and 72 °C for 5 min; and a final cycle at 94 °C for 60 s, 45 °C for 2 min, and 72 °C for 5 min. When exact primers were used, the sample was heated by 2 min at 94 °C, followed by 33 cycles at 94 °C for 60 s, 60 °C for 90 s, and 72 °C for 60 s and a final cycle at 94 °C for 60 s, 60 °C for 90 s, and 72 °C for 5 min.

**Cloning and Sequencing of the α-Subunit—** Aliquots (one-tenth to one-twenty-five) of the purified cDNA pools prepared from mRNAs isolated from pigeon liver and breast muscle were used. The initial PCR product of 605 bp was obtained using a sense primer (5'-CCTATCTTTAATAGCTTGAGAGG-3') corresponding to the peptide sequence PVFNTVKE that is found in the rat and human proteins (GenBank™ accession numbers J03621 and Z68204) and an antisense primer (5'-GRTGNCCAT-3') for the peptide sequence MGHA, which is conserved in procaryotes and eucaryotes and contains the active-site histidine residue of the N-ε (e.coli) (18). The 605-bp sequence was used to design exact primers for use in 3'-RACE; the sense primer was 5'-GAAATGCGAGGAAATGTCTG-3' (corresponding to the peptide sequence EIGGNAE), which was used with a 3'-RACE adaptor primer (5'-CGTCAATGACGATCCGACGCAGTCCATTAG-3') to obtain a 504-bp product. A PCR product of 414 bp that encoded the N terminus of the mature α-subunit was obtained using a sense primer of 5'-CTCTCTCGACAACAGAATGGG-3', corresponding to the peptide sequence FVFTVKE that is found in the rat and human proteins, and an antisense primer containing the gene-specific sequence of 5'-CAGTAGTGAGATCTAGTCTG-3' (corresponding to QDRTRLV). The sequence that encodes the mature protein was obtained by overlapping the three products and was confirmed by double-sided sequencing of each individual product using gene-specific primers.

**Cloning and Sequencing of the β-Subunits in Pigeon—** The primers used are listed in Table I. The A-β sequence was obtained by RT-PCR methods using mRNA from pigeon breast muscle. An initial PCR product of 217 bp was amplified using primer LAGG, which corresponds to the highly conserved sequence LAGGRRGK found in several β-subunits, in combination with a mixture of the overlapping primers IFEE and MIFE, both of which were designed for the A-β peptide EYYFAITMER. The sequence thus obtained was used to design exact primers for use in extending the sequence in both the 3' and 5' directions. Primer BBR1 was used with an oligo(dT) adaptor primer to obtain a 1762-bp product by 3'-RACE. Following sequencing of this product, exact internal primers were designed and used to amplify and sequence smaller products. To obtain the N-terminal region of the mature protein, primer CAR6 was designed based on the conserved sequence QVQQQ located in the mitochondrial targeting sequence as shown in mouse and human ESTs. This primer was used in combination with the exact primer RVRLA to obtain a PCR product of 552 bp.

| Primer name | Sequence |
|-------------|----------|
| LAGG        | TTAGCGGAGNAGGAGGAAGG |
| IFEE        | GAGGATCCATTCGCGGAAAG |
| MIFE        | GAGGATCCATTCGCGGAAAG |
| BBR1        | GAAGAGCAAAACTGCTTCCCTCAAG |
| CAR6        | GARTGACACCARCARCAR |
| RVRRA       | TCCCAATCTTGCGGGAACCAAC |
| VNGNA       | CNARNCCNCGCCNCTT |
| NGAGL       | NRCNARNCCNCGCCNCTT |
| L1VB2       | AGGGCAAGTTGTGTTGTTG |
| PLPX        | TGAATAACCTACGGAGATGTC |
| RWNL        | GATGCGTGACGACGC |
| BK3R        | GATGACCTCTCGCCAAATCC |
| HMB         | CAGGTTCTGGGAAATTTGTTG |
| HABSP       | ATGATATAGACATTTACAAAC |
| MABSP       | CAGTATAAAAGAATAGCAAC |
| PIGN        | TTAACACCATGAGCTCCCAAATAC |
| PIG5A       | CATGTCATAGGATACTTCCACAC |
| PIG3R       | ITTTCCACCATGATGGAATTC |
Preliminary studies were carried out to optimize each reaction while maintaining nonsaturating conditions. Thus, for each reaction, not more than two additional cycles would be needed to double the amount of product. Two-step PCR cycles were used in which annealing occurred at 66°C (GAPDH) or 60 °C (SCS messages), and extensions were carried out for 1.5 min. The amplified regions were 700 base pairs. Primers were designed for regions that share no significant homology between the A- and G- isoforms.

For expression studies in mouse, total RNAs from 10 tissues were purchased from Ambion Inc. Pools of cDNA were prepared from 1 mg/ml samples of total RNA as described above. Before carrying out expression studies, the set of cDNAs was normalized with primers made specific for β-actin using a primer in the 3′-untranslated region that differs from other actin isoforms. The protocols were otherwise similar to those used in the human tissue study. The number of cycles used for β-actin and the α- and two β-subunit sequences of SCS are listed in the legend to Fig. 4. Each primer pair used in the mouse and human studies was designed for regions that share no significant homology between the A- and G-β isoforms. The primers were also designed to be noncomplementary and to have similar Tm values.

**RESULTS**

**Cloning and Sequencing of the α-subunit**—Prior characterization studies showed that the same α-subunit was present in G-SCS purified from pigeon liver and A-SCS from breast muscle (15). Using RT-PCR and three pairs of primers, the same mRNA sequence for the α-chain was reverse-transcribed, cloned, and sequenced in breast muscle and liver (see “Experimental Procedures”). Assuming that cleavage of the signal peptide occurs at the same location in pigeon as in rat (25), the computed molecular mass and pI of the 306-residue processed α-subunit in pigeon are 32,051 Da and 9.1.

The deduced amino acid sequence of the α-subunit in pigeon is aligned with homologous sequences in Fig. 1. Sequences from species other than pigeon were obtained from GenBank™ as follows: rat (J03621), pig (AF008588), E. coli (J01619), C. elegans (P53596), Dictyostelium discoideum (U23408), yeast (Saccharomyces cerevisiae; P53598), Thermus aquaticus (X12815), T. vaginalis (L31929), and Arabidopsis thaliana (X69138). The sequences shown are the portions aligning with the mature protein in rat (25). The His that is located in the active site is indicated by an asterisk.

**Expression of Succinyl-CoA Synthetases**

Manual PT3158-1 (32). Preliminary studies were carried out to optimize each reaction while maintaining nonsaturating conditions. Thus, for each reaction, not more than two additional cycles would be needed to double the amount of product. Two-step PCR cycles were used in which annealing occurred at 66 °C (GAPDH) or 60 °C (SCS messages), and extensions were carried out for 1.5 min. The amplified regions were 700 base pairs. Primers were designed for regions that share no significant homology between the A- and G-β isoforms.

For expression studies in mouse, total RNAs from 10 tissues were purchased from Ambion Inc. Pools of cDNA were prepared from 1 μg/μl samples of total RNA as described above. Before carrying out expression studies, the set of cDNAs was normalized with primers made specific for β-actin using a primer in the 3′-untranslated region that differs from other actin isoforms. The protocols were otherwise similar to those used in the human tissue study. The number of cycles used for β-actin and the α- and two β-subunit sequences of SCS are listed in the legend to Fig. 4. Each primer pair used in the mouse and human studies was designed for regions that share no significant homology between the A- and G-β isoforms. The primers were also designed to be noncomplementary and to have similar Tm values.
facilitative residue in E. coli (27). This residue is conserved in the vertebrate sequences, but not in three of the other species shown.

Sequences of the β-Subunits of G-SCS and A-SCS in Higher Vertebrates—From the tryptic digests of A-SCS and G-SCS, one peptide was selected from each for sequencing. The sequences determined were EYYFAITMER for A-SCS and ETYFAILMDR for G-SCS. Both sequences align with ETYLAILMDR for G-SCS in pig. Fortuitously, the two peptides chosen are from the same region of their respective subunits (residues 116–125 in Fig. 1). Degenerate oligonucleotide primers for these peptides and conserved regions in known sequences of β-subunits were used to obtain initial PCR products. The pools of cDNA were prepared from mRNAs of tissues where each isoform is predominantly expressed: liver for the β-subunit of G-SCS and breast muscle for the β-subunit of A-SCS. The deduced amino acid sequences of the G- and A-β subunits in pigeon are shown in Fig. 2. The two sequences are 53% identical at the amino acid level.

The nucleotide sequences of the two β-subunits were used to probe the publicly accessible data banks of ESTs. Several homologous ESTs were identified that fell into two groups: one group showing at least 90% identity to A-β in pigeon and the other displaying similar identity to G-β. As described under

Fig. 2. Alignment of the amino acid sequences of the β-subunits of A-SCS and G-SCS from five eucaryotic species. The accession numbers for the sequences shown are listed in Table II. The sequences are for the mature proteins, assuming that the signal peptides are cleaved at the same position as in G-β in pig (22).

Sequences of the β-Subunits of G-SCS and A-SCS in Higher Vertebrates—From the tryptic digests of A-SCS and G-SCS, one peptide was selected from each for sequencing. The sequences determined were EYYFAITMER for A-SCS and ETYFAILMDR for G-SCS. Both sequences align with ETYLAILMDR for G-SCS in pig. Fortuitously, the two peptides chosen are from the same region of their respective subunits (residues 116–125 in Fig. 1). Degenerate oligonucleotide primers for these peptides and conserved regions in known sequences of β-subunits were used to obtain initial PCR products. The pools of cDNA were prepared from mRNAs of tissues where each isoform is predominantly expressed: liver for the β-subunit of G-SCS and breast muscle for the β-subunit of A-SCS. The deduced amino acid sequences of the G-β and A-β subunits in pigeon are shown in Fig. 2. The two sequences are 53% identical at the amino acid level.

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Expression of Succinyl-CoA Synthetases

**TABLE II**

| Sequence | Accession No. | Human A-β | Human G-β |
|----------|---------------|-----------|-----------|
| Human A-β | AF058953     | 100       | 53        |
| Pig A-β   | AF061966     | 95        | 53        |
| Mouse A-β | AF058955     | 92        | 54        |
| Pigeon A-β| AF043540     | 89        | 54        |
| C. elegans SCB1 | P53558 | 61    | 57        |
| N. frontalis | P53557 | 59    | 57        |
| Human G-β | AF058954     | 53        | 100       |
| Pig G-β   | P53590       | 52        | 94        |
| Mouse G-β | AF058966     | 52        | 94        |
| Pigeon G-β| AF043541     | 53        | 89        |
| C. elegans SCB2 | P53589 | 50    | 54        |
| Yeast (S. cerevisiae) | P53312 | 47  | 47        |
| T. vaginalis | Q05184 | 48    | 48        |
| E. coli   | P07480       | 46        | 45        |
| Caenorhabditis burnetii | P53592 | 48    | 45        |
| Haemophilus influenzae | P45101 | 44    | 48        |
| Bacillus subtilis | C69005 | 47  | 41        |
| T. aquaticus | P25126 | 41    | 44        |
| Mycobacterium tuberculosis | P73539 | 39  | 39        |
| Methanococcus jannaschii | Q57683 | 39  | 42        |
| Methanobacterium thermoautotrophicum | E09719 | 38  | 39        |

a Data banks and accession numbers are as follows: those beginning with P or G, Swiss-Prot; AF, GenBank; and C or E, PIR. The matching percentages were based on the alignment window as calculated by DNASIS (Version 2.5).

The sequences shown in Fig. 2 are for the mature β-subunits based on the site of cleavage that has been determined for G-β in pig. Alignment of all known sequences (not shown, but the species are listed in Table II) indicates that the initiator methionine of bacterial sequences aligns with the first residue (leucine) of the processed eucaryotic β-subunits. The mature A-β and G-β subunits in vertebrates are composed of 411 and 395 residues, respectively. In human, the calculated masses of A-β and G-β are 44,589 and 42,653 kDa, and their theoretical pI values are 5.50 and 5.25, respectively.

The tissue sources of the available ESTs suggest that the ATP-specific β-subunit is expressed in numerous tissues in mouse and human. This was confirmed using RT-PCR to screen 10 mouse and 16 human tissues. The results are shown in Figs. 4 and 5. The study provided an indication of the levels of expression of the α- and both β-subunits with that of β-actin in mouse and GAPDH in human. In judging the results with human, it should be noted that tissues with fewer mitochondria and thus less reliant on oxidative metabolism can be expected to give much weaker bands for the SCS subunits relative to GAPDH, a glycolytic enzyme.

The level of total β-subunits usually mirrors that of the α-subunit. In mouse, expression of A-β is strongest in liver, heart, brain, and testis and clearly exceeds that of G-β in brain and testis. The bands for G-β resulted from two more cycles than used for A-β. The tissues that most strongly express G-β are liver, kidney, and heart, with the level in kidney perhaps exceeding that of A-β. In human, A-β is expressed at a high level in kidney, heart, brain, prostate, testis, ovary, skeletal muscle, small intestine, and spleen. G-β expression was clearly stronger than A-β expression in liver. Other tissues in which it is strongly expressed include kidney and heart. Expression of G-β appeared weaker than that of A-β in prostate and skeletal muscle.

**DISCUSSION**

The work presented in this and the preceding paper (15) clearly establishes that genes for two forms of succinyl-CoA synthetase differing in nucleotide specificity are expressed in vertebrate species. We reported that the ratio of enzymatic activities in pigeon tissues varies widely, with at least 99% of SCS activity in liver being GTP-specific, whereas the ATP-specific form similarly predominates in breast muscle (15). Characterization of both forms of SCS indicated that the same α-subunit was incorporated. Therefore, nucleotide specificity must be conferred by the β-subunit (15).

The results of enzyme purification and characterization of A-SCS and G-SCS in pigeon (15) and G-SCS in pig (22) were
Expression of Succinyl-CoA Synthetases

ing nucleotide specificity. Assuming that some of these residues must be fully conserved in G-SCS, but differ from their equally conserved counterparts in A-SCS. Some of these residues must be more abundant than G-SCS, 32.

The alignment in Fig. 2 reveals a number of residues that are generally sufficient to visualize transcripts of medium abundance. These regions provide different hydrogen-bonding potentials, for example, residue 251, which is asparagine in the A-β isoforms and lysine in the G-β forms.

Both the A- and G-forms of the β-subunit show high homology when compared within their group. Earlier suggestions that the α-subunit is more highly conserved than the β-subunit (8, 13, 22) were based on comparisons of sequences of β-subunits that we now know to vary in their specificity. When β-subunits of the same specificity are compared, identity of sequence is as high as found in α-subunits. However, within a given species, the two types of β-subunits share only ~50% sequence identity. From alignment of sequences, it is concluded that regions of strict conservation occur throughout the sequences of both types. Conserved regions that are shared in common between the A- and G-type subunits may be involved in binding the substrates they share in common. Also, both β-subunits are further restrained to interact with the same α-subunit. Regions that are conserved within a subunit type, but not between subunits, could be a reflection of interactions with other proteins. In this regard, there is evidence for complex formation between G-SCS and α-ketoglutarate dehydrogenase (26) and between SCS and nucleoside-diphosphate kinase (29, 30).

To screen tissue expression of the β-subunits, we used PCR-based methods with normalized cDNA pools. This approach permits detection of transcripts of various levels of abundance and allows differences in tissue expression to be determined. The PCR method can be used to reliably distinguish message levels for related isoforms, even when there are small differences in isoform sequence.

The expression studies indicate that the A-β transcript is more abundant than G-β in most tissues. In comparing the results in human (Fig. 5), it should be noted that tissue differences in the relative amounts of GAPDH, which is considered to be an abundant transcript, correlate well with normalized Northern blot panels (CLONTECH User Manual PT3158-1 (32)). The bands shown for the α-subunit and G-β were produced by 32 cycles, whereas 30 were used for A-β. Even with two fewer PCR cycles, the bands for A-β are generally stronger than those for G-β. Thus, the transcript for A-β is present at a fairly high level in most tissues. The CLONTECH User Manual (32) indicates that when using Taq polymerase, 30–35 cycles are generally sufficient to visualize transcripts of medium abundance.

The expression of A-β is strongest in tissues that are more highly dependent on oxidative metabolism for ATP production (heart, brain, and skeletal muscle), whereas G-β is strongest in tissues that are highly involved in biosynthesis, for example, gluconeogenesis. This is consistent with the role of G-SCS being to generate GTP for specialized functions.

Although the work presented here indicates that the level of mRNA for A-β exceeds that of G-β in nearly all tissues, preliminary data suggest that the relative levels of these transcripts are not directly related to relative enzymatic activities. Enzyme assays of eight mouse and four rat tissues indicated

![Fig. 4. Relative levels of transcripts of the subunits of SCS and β-actin in 10 mouse tissues as demonstrated by RT-PCR. The procedures used are described under “Experimental Procedures.” The number of cycles used were as follows: β-actin, 24; α-subunit of SCS, 25; β-subunit of A-SCS, 25; and β-subunit of G-SCS, 27.](image-url)

![Fig. 5. Relative levels of transcripts of the subunits of SCS and β-actin in 16 human tissues as demonstrated by RT-PCR. The procedures used are described under “Experimental Procedures.” The number of cycles used were as follows: GAPDH, 26; α-subunit of SCS, 32; β-subunit of A-SCS, 30; and β-subunit of G-SCS, 32.](image-url)
that G-SCS is usually the predominant enzyme in mammals,\(^2\) with apparent G-SCS activity exceeding that of A-SCS by 10-fold in extracts of the particulate fractions of kidney and liver. Even in mouse testis, where the transcript for G-\(\beta\) appears at best to be rare, enzymatic activity is predominantly G-SCS. Reliable assessment of a relatively minor amount of A-SCS (or G-SCS) is difficult regardless of tissue because the apparent activity may arise from the other form of SCS and the recycling of endogenous nucleotides by nucleoside-diphosphate kinase (29). However, both enzymes are clearly present in heart and brain since their apparent activities are additive when both nucleotides are present in the assay. In the case of rat heart, the two activities have been separated by chromatography.\(^2\) The above results are in general agreement with those of Weitzman et al. (12), who first reported the occurrence of A-SCS in mammals. Our conclusions are based on high performance liquid chromatography-based assays (31) that easily quantify micromolar changes in CoA and succinyl-CoA in assays in which guanine, adenine, or both nucleotides are included. Both directions of catalysis have been measured. More definitive studies are clearly needed that will correlate enzyme assays of A-SCS and G-SCS with the amounts of the relevant mRNAs and \(\alpha\)- and \(\beta\)-subunits.

The presence of both types of \(\beta\)-subunit within the same subcellular compartment, if this occurs, would suggest the possibility of a regulatory mechanism in which G-\(\beta\) competes with A-\(\beta\) for binding to the \(\alpha\)-subunit and thus constitutes of the active enzyme. Regulation and integration of the adenine and guanine nucleotide pools within mitochondria could be achieved by a wide variety of mechanisms, including regulation of transcription, translation, or protein-protein interactions.

The phylogenetic tree presented in Fig. 3, together with biochemical data regarding SCS specificity in nonvertebrates (11), suggests that the gene duplication that led to ATP- and GTP-specific succinyl-CoA synthetases occurred before the evolution of \(C.\) elegans. The strong conservation within the A-\(\beta\) and G-\(\beta\) groups, together with the widespread tissue expression of each form, further suggests that A-SCS and G-SCS each play an important metabolic role. The nucleotide specificity of the long known G-SCS in animals has been a matter of curiosity. Its evolution cannot be a trivial happenstance. Although ATP and GTP have identical standard free energies of hydrolysis, their concentrations in any cellular compartment are very different. Perhaps more important, the ATP/ADP and GTP/GDP ratios may be very different, which has strong thermodynamic implications for which nucleotide is used in a given metabolic reaction. In the past 20 years, much has been learned about the selective use of particular nucleotides in various biological phenomena. Thus, GTP is used in signaling (G-proteins and the small GTPases) and protein translation, UTP in glycogen synthesis, and CTP in lipid biosynthesis. With the present knowledge that cells in all but the more primitive eucaryotes can express either or both A-SCS and G-SCS, the time has come to probe deeper into the metabolic significance of the nucleotide specificity of these enzymes.

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\(^2\) D. O. Lambeth, unpublished data.