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Steroid Receptor Coactivators: Servants and Masters for Control of Systems Metabolism

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Abstract

Coregulator recruitment to nuclear receptors (NRs) and other transcription factors is essential for proper metabolic gene regulation with coactivators enhancing and corepressors attenuating gene transcription. The Steroid Receptor Coactivator (SRC) family is composed of three homologous members (SRC-1, SRC-2, and SRC-3), which are uniquely important for mediating steroid hormone and mitogenic actions. An accumulating body of work highlights the diverse array of metabolic functions regulated by the SRCs, including systemic metabolite homeostasis, inflammation, and energy regulation. Here we discuss the cooperative and unique functions among the SRCs to provide a comprehensive atlas of systemic SRC metabolic regulation. Deciphering the fractional and synergistic contributions of the SRCs to metabolic homeostasis is critical to fully understand the networks underlying metabolic transcriptional regulation.

Keywords

Steroid Receptor Coactivator; Metabolism; Nuclear Receptor; Transcription; Coregulator

The Fundamentals of SRCs

With over 450 coregulators identified to date, the SRCs offer tremendous flexibility in transcriptional regulation and gene expression (Box1). The three family members, SRC-1 (NCOA1), SRC-2 (NCOA2/Grip1/Tif2), and SRC-3 (NCOA3/p/CIP/AIB1/ACTR/RAC3/TRAM-1) belong to the structurally homologous p160 family of coactivators. The most conserved domain is the amino-terminal basic helix-loop-helix-Per/ARNT/Sim (b-HLH-PAS) that facilitates protein-protein interactions with other coregulators and transcription factors (TFs) and contains a canonical nuclear localization signal [1–3]. More centrally located is a serine/threonine-rich domain, the phosphorylation status of which influences SRC activity. Following is a receptor and TF interaction domain containing three LxxLL (x any amino acid) motifs that facilitate NR binding. Near the carboxyl-terminus are two

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activation domains (AD1 and AD2) that interface with other coregulators such as CBP, p300, CARM1, and PRMT1.

The SRCs are involved in the regulation of virtually all aspects of gene expression including transcriptional initiation, cofactor recruitment, elongation, RNA splicing, posttranslational modification of NRs/coregulators, and translation. Given the ubiquitous expression of the SRCs, it is not surprising that their ablation or overexpression is directly linked to various disease outcomes. Nowhere is this fact more evident than in considering the breadth of SRC-centric literature supporting their established roles in reproductive, developmental and cancer biology [2,4–11]. More specifically, SRC-1 has been shown to interact with estrogen receptor (ER) and progesterone receptor (PR) to regulate uterine function with loss of SRC-1 decreasing uterine growth [4]. SRC-1 is an important regulator in the progression of endometriosis [5]. In breast cancer, SRC-1 is an oncogene associated with poor outcome prognosis with roles in cell migration and metastasis [6]. Loss of SRC-2 affects whole body physiology with animal growth retardation and reduced adiposity [7]. Defects in spermatogenesis and testicular degeneration are observed in *SRC-2* null adult males [7]. SRC-2 also plays a role in cancer development and is amplified in prostate cancer and translocations of *SRC-2* with monocytic leukemia zinc finger protein (MOZ) have been found in cases of acute myeloid leukemia [8,9]. Similarly, *SRC-3* ablation in mice results in decreased growth, development, and reduction of circulating estrogen that delays puberty [10]. As an oncogene, SRC-3 has been extensively studied in various cancers but mostly as an amplified gene in breast cancer with defined molecular roles in cell growth, EMT, and Her2 signaling [2,11]. However, in addition to their developmental and proliferative functions, the SRCs are emerging as key pleiotropic regulators of metabolism. While numerous reviews underscore the importance of the SRCs in endocrine responsive tissues and cancers, there remains a deficit of reviews focusing on the collective potency of the SRCs in maintenance of metabolic programs. The SRCs regulate diverse metabolic processes in several key tissues including the brain, liver, heart and skeletal muscle, as well as brown and white adipose (BAT and WAT) (Figure 1). This review is designed to highlight the unique tissue- and pathway-specific functions by which SRCs regulate metabolism.

Lipid metabolism

White and Brown Adipose Tissue

SRC regulation of lipid metabolism is complex and largely deduced using congenic mouse knockout models. Each of the SRCs displays unique roles in lipid metabolism including fatty acid biosynthesis, catabolism, and adipogenesis. Overall, the *SRC-1*^{-/-} mouse exhibits decreased energy expenditure resulting in increased adiposity, and both *SRC-2*^{-/-} and *SRC-3*^{-/-} mice are lean and resistant to obesity upon challenge with a high-fat diet (HFD) [12,13]. Interestingly, a HFD alters the ratio of SRC-1 and SRC-2 protein expression in BAT and WAT by increasing SRC-2 and decreasing SRC-1 expression [12]. The counterbalance of SRC regulation may be a consequence of SRC-1 and SRC-2 both competing for binding to peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α regulator of mitochondrial biogenesis and function, and PPARγ, a NR that

controls adipogenesis and regulates fatty acid storage and glucose metabolism. Fatty acids serve as ligands for the PPARs and stimulate complex formation with SRC-1; however, fatty-acyl-CoAs inhibit recruitment of SRC-1 to the ligand binding domain (LBD) of PPAR γ and its activation, thus possibly shutting down lipolysis and adipogenic programs in adipose cells [14]. In the BAT of mice on a HFD, SRC-2 decreases the stability of the PGC1 α /PPAR γ complex thereby reducing adaptive thermogenesis while increasing energy storage and adiposity. In the presence of fatty acids, SRC-1 strengthens the integrity of the PGC1 α /PPAR γ complex and, in an *SRC-2* null background, decreases lipolysis in WAT while decreasing thermogenesis and energy expenditure in BAT [12]. In addition to fatty acids regulating SRC-1 expression, increasing age decreases SRC-1 expression in the WAT of mice and humans [15]. This decrease in SRC-1 expression may be partially responsible for the general decrease in insulin sensitivity and increase in adipogenesis observed with aging.

As with *SRC-2* ablation, loss of SRC-3 increases basal metabolism and mitochondrial function in BAT via expression of GCN5, an acetyltransferase for PGC1 α [13]. Reciprocal SRC-3 and GCN5 expression adjusts PGC1 α activity, as GCN5 inhibits PGC1 α mitochondrial actions through its acetylation and increases energy expenditure to protect against obesity. As a result, *SRC-3*^{-/-} mice display improved adaptive thermogenesis and lower respiratory quotients indicating that the primary fuel source of *SRC-3*^{-/-} mice is lipid oxidation [13]. Diet composition also regulates SRC-3 expression with HFD increasing SRC-3 expression in adipose, whereas caloric restriction decreases its expression.

Interestingly, ablation of both *SRC-1* and *SRC-3* in mice (*SRC-1/3*^{DKO}) confers resistance to HFD-mediated obesity [16]. Much like *SRC-1*^{-/-} mice, *SRC-1/3*^{DKO} mice exhibit decreased thermogenesis. However, unlike *SRC-1*^{-/-} mice, decreased thermogenesis is a consequence of impaired BAT development resulting from reduced expression in PPAR γ target genes that are involved in both adipogenesis and mitochondrial uncoupling. Consequently, *SRC-1/3*^{DKO} mice have an increased metabolic rate, which correlates with increased food consumption on HFD [16]. Adipogenesis defects from SRC-3 loss are attributable to decreased PPAR γ expression [17]. SRC-3 drives the adipogenic gene program by interacting with Ccaat-enhancer-binding proteins (C/EBP) on the PPAR γ promoter. Either SRC-2 or SRC-3 loss impairs lipogenesis by decreasing the expression of PPAR γ and C/EBP target genes including *ADFP*, *SREBP1c*, and *FASN* [17]. While important in adipogenesis, SRC-2 and SRC-3 levels were not altered during adipocyte differentiation. Only SRC-1 expression changed during differentiation, suggesting an important divergence of coactivator function in lipid metabolism and adipocyte differentiation [17].

Within adipose, SRC-1 increases adaptive thermogenesis to promote energy usage. Oppositely, SRC-2 and SRC-3 promote adipogenic energy conservation and storage as well as adipogenesis. The *SRC-1/3*^{DKO} mice show that SRC-3 largely regulates thermogenesis through coactivation of PPAR γ and C/EBP and increased transcription of their target genes in adipogenesis. Additionally, nutrient status affects the adipogenic levels of the SRCs. SRC-1 decreases in expression with a HFD whereas both SRC-2 and SRC-3 increase in expression, correlating with their roles as coactivators that promote energy storage and adipogenesis (Figures 1 and 2).

Liver

The SRCs also regulate lipid metabolism and metabolite homeostasis in the liver. *SRC-1* ablation impacts hepatic metabolites, particularly in the fed-to-fasted state, by increasing acyl carnitines, an effect that is not observed in mice devoid of *SRC-2* or *SRC-3*. The collective increase in acyl carnitines in both liver and plasma suggests an important role for *SRC-1* in regulating hepatic β -oxidation [18].

Quantification of fatty acid metabolites upon *SRC-2* ablation revealed few alterations during homeostatic conditions [18]. However, when analyzed as a function of diurnal variation, loss of *SRC-2* dynamically influences fatty acid metabolism [19]. These findings are congruent with published roles for *SRC-2* in facilitating hepatic fat absorption [20]. *SRC-2* coactivates the bile acid receptor farnesoid X receptor (FXR) in the liver to promote transcription of *BSEP*, the gene encoding the bile Salt Export Pump transporter protein, which is essential for bile acid transport and dietary fat absorption. As a result, *SRC-2* deletion increases fecal triglycerides but decreases circulating plasma triglycerides and hepatic bile acid accumulation with reduced expression of bile acid synthesis genes *Cyp7a1*, *Cyp8b1*, *Cyp7b1*, *Cyp27a1*, and *Ntcp* [20].

As observed in adipose tissue, hepatic *SRC-3* expression increases upon HFD feeding [21]. Loss of *SRC-3* protects against HFD-induced hepatic steatosis by reducing lipid accumulation and the accompanying inflammatory response. F4/80-staining reveals decreased macrophage infiltration and inflammatory gene expression (*Cyp2e1*, *IP-10*, *Mcp1*, and *Mip1a*) in the liver of *SRC-3*^{-/-} mice [21]. *SRC-3* ablation leads to an increase in *PPAR α* expression as well as genes involved in β -oxidation (*Aco1*, *Cpt1a*, *Cpt1b*, and *Scad*) resulting in increased serum β -hydroxybutyrate [21]. *SRC-3* coactivates retinoic acid receptor alpha (RAR α) in a ligand-dependent fashion whereby the RAR α ligand all-trans retinoic acid (ARTA) promotes the expression of the NR chicken ovalbumin upstream promoter transcription factor II (*COUPTF-II*), which in turn represses *PPAR α* expression, thus leading to decreased hepatic β -oxidation [21]. In humans, *SRC-3* expression positively correlates with hepatic fibrosis and reduced hepatic injury [22]. Likewise, loss of *SRC-3* reduces carbon tetrachloride-induced hepatic fibrosis due to a suppressed inflammatory response that results from attenuated TGF β 1/Smad signaling [22].

When taken together, each SRC regulates hepatic lipid levels. In summary, *SRC-1* functions in the fed to fasted transition as a coactivator regulating lipid flux, *SRC-2* promotes fat absorption through *BSEP*, and *SRC-3* regulates the inflammatory response and increases β -oxidation to decrease hepatic lipid accumulation.

Muscle

Muscle-specific ablation of *SRC-2* (*SRC-2*^{(i)skm-/-}) drives mitochondrial uncoupling and protects against HFD-induced obesity characterized by decreased muscle mass, thereby reducing fat accumulation [23]. The increased energy expenditure in *SRC-2*^{(i)skm-/-} mice was accompanied by a shift from lipid usage to carbohydrate consumption. Metabolic shifts are the consequence of *SRC-2* expression that inversely affects *SRC-1* expression. *SRC-1* and *SRC-2* reciprocally regulate common metabolic programs in muscle. As an example,

SRC-2 ablation increases the expression of mitochondrial uncoupling protein 3 (*UCP3*), which affects energy metabolism by promoting energy dissipation as heat, whereas loss of *SRC-1* decreases *UCP3* expression. These changes alter mitochondrial oxidative phosphorylation in skeletal muscle and explain the fuel source switch. However, the muscle-specific double knockout of *SRC-1* and *SRC-2* resulted in no difference in *UCP3* expression, body temperature, energy expenditure, fatty acid metabolism or mitochondrial oxidative phosphorylation gene expression, suggesting that up-regulation of *SRC-1* in skeletal muscle, an effect of *SRC-2* ablation, is partly responsible for the phenotypes observed in *SRC-2^{(i)skm}-/-* mice.

SRC-3 regulates long-chain fatty acid metabolism specifically by maintaining the expression of carnitine-acyl carnitine translocase (*CACT*) in skeletal muscle [18]. Phenotypes mirroring *CACT* deficiency are observed in *SRC-3*^{-/-} mice including accumulation of long-chain fatty acids, reduced cardiac and skeletal muscle performance, neurological defects, hypoglycemia, hyperammonemia, and hypoketonemia [24]. Interestingly, the hypoglycemic and endurance phenotypes of the *SRC-3*^{-/-} mice are partially rescued by feeding a short-chain fatty acid diet and exacerbated by a diet rich in long-chain fatty acids [24].

As seen in both WAT and BAT, *SRC-1* promotes energy expenditure with *SRC-2* oppositely regulating energy storage programs in the muscle. *SRC-3* alternatively regulates fuel source accessibility in the muscle affecting overall muscle endurance.

Collectively, the multifunctional impact of the *SRCs* on lipid metabolism in various metabolic tissues suggests that they may have evolved for the primary purpose of coordinating utilization or storage of fat (Figure 2). Specifically, this counterbalance is achieved by *SRC-1* promoting lipid utilization while *SRC-2* and *SRC-3* regulating fat storage.

Carbohydrate Metabolism

The *SRCs* function in various elements of carbohydrate metabolism including glycolysis/gluconeogenesis, the TCA cycle, and the insulin response. Analysis of gene expression from hepatic microarrays following ablation of each *SRC* family member revealed clear metabolic roles for *SRC-1* and *SRC-2* in carbohydrate metabolism [25]. More specifically, pathway analysis of genes influenced by the loss of the *SRCs* revealed that *SRC-1* ablation repressed gene expression for processes involved in energy consumption, which is consistent with the increased adiposity of *SRC-1*^{-/-} mice [12,25]. *SRC-1* is an important mediator of gluconeogenesis in the fed-to-fasted metabolic transition. Under fasting conditions, hepatic *SRC-1* expression is upregulated and induces gluconeogenesis by coactivating CEBP α and increasing transcription of its target gene pyruvate carboxylase (*PC*) [26]. Administering either glucose or insulin, both of which represent carbohydrate signaling molecules, decreases *SRC-1* expression, whereas glucose deprivation increases *SRC-1* protein stability by reducing its 26S proteasomal degradation via altered NAD⁺/NADH ratios, metabolites that underlie *SRC-1*'s response to glucose deprivation [26,27].

SRC-3 is a key regulator of the insulin signaling pathway where it affects carbohydrate utilization in both fasted and re-fed conditions, thus resulting in modest hypoglycemia

[13,24]. *SRC-3*^{-/-} mice have reduced levels of circulating insulin-like growth factor 1 (IGF-1) as a result of reduced serum levels of its carrier, IGF-binding protein 3 (IGFBP3) [28,29]. Contrary to the *SRC-3*^{-/-} mouse phenotype, specific genetic disruptions of posttranslational modifications (PTM) in SRC-3 yields mice with a phenotype consistent with metabolic syndrome; increased bodyweight, increased adiposity, insulin resistance, and age-dependent hyperglycemia in both fasting and *ad libitum* feeding conditions [30]. These loss of function mutations at sites of PTM of SRC-3 increase transcription of *IGFBP3* and elevate plasma levels of IGFBP3, which increase IGF-1 signaling [30]. Additionally, *SRC-1/3*^{DKO} increases insulin sensitivity and glucose metabolism *in vivo* and in a cell-autonomous manner [31]. However, when challenged with a HFD or upon aging, *SRC-1/3*^{DKO} mice remain insulin sensitive and glucose tolerant [31]. Likewise, *SRC-3*^{-/-} mice are insulin sensitive with greater glucose clearance when fed both normal chow or HFD; however, *SRC-1*^{-/-} mice are neither insulin sensitive nor exhibit increased insulin secretion [13,26].

Hepatic microarray analysis reveals the involvement of SRC-2 in gene programs associated with increased energy expenditure and decreased energy accretion, consistent with the decreased adiposity observed in the *SRC-2*^{-/-} mice [7,25]. Changes in gene expression resulting from *SRC-2* ablation enrich for processes involved in fatty acid degradation, gluconeogenesis/glycolysis, and glycogen storage [25]. Cistronic analysis of SRC-2 chromatin occupancy during two phases of the diurnal cycle reflects the involvement of SRC-2 in carbohydrate metabolism during the light phase, and SRC-2 occupancy enriches in processes involving starch and sucrose metabolism [19]. Additionally, SRC-2 loss in the liver alters circadian cycling of metabolites involved in glycolysis and gluconeogenesis [19]. Focusing on gluconeogenesis, *SRC-2*^{-/-} mice phenocopy von Gierke's disease with fasting hypoglycemia and reduced expression of glucose 6-phosphatase (*G6PC*), the gene encoding the rate-limiting enzyme for glucose release in the liver [32]. SRC-2 regulates fasting blood glucose by coactivating RAR-related orphan receptor (ROR) on the *G6PC* promoter [32]. SRC-2 regulation of *G6PC* also has implications in liver cancer, as decreased SRC-2 and *G6PC* expression are associated with poor patient survival [33]. Further supporting these data, SRC-2 is a hepatic tumor suppressor in mice; *SRC-2*^{-/-} mice show increased tumorigenesis in a carcinogen-induced model for liver cancer [33]. In addition to the liver, SRC-2 is a regulator of carbohydrate metabolism within endothelial stem cells (ESCs) and in the heart [34,35]. SRC-2 is required in ESCs to maintain the glycolytic flux necessary for the development of decidualization such that loss of SRC-2 decreases key rate-limiting glycolytic enzymes [34]. Likewise, SRC-2 loss in the mouse heart decreases glycolytic gene expression mirroring expression changes observed in human patients suffering from cardiac pressure overload and failure [35]. Reinforcing the importance of SRC-2 in the cardiac stress response, decreased SRC-2 expression has been identified in human patients experiencing heart failure [36].

In total, all of the SRCs uniquely contribute to carbohydrate utilization and storage. SRC-1 mediates the anabolic process of gluconeogenesis during fasting conditions. SRC-2 regulates fasting utilization of glucose from glycogen stores. Finally, SRC-3 governs glucose uptake by serving as a mediator of insulin signaling (Figures 2 and 3).

Amino Acid Metabolism

Amino acid metabolism is fundamental to protein translation and transcription, and all three SRCs impact amino acid homeostasis. Amino acids are key precursors for gluconeogenesis, neurotransmitter biosynthesis, and anaplerotic metabolism. SRC-1, a regulator of gluconeogenesis, also regulates amino acid metabolism in the liver through maintenance of tyrosine aminotransferase (*TAT*) gene expression, which, in turn, alters tyrosine levels. Hepatic SRC-1 ablation also increases other amino acid profiles including aspartic acid, glutamic acid, methionine, and phenylalanine, further indicating that SRC-1 is an important mediator of amino acid metabolism (unpublished data) [18]. SRC-2 affects amino acid composition specifically in the heart by altering profiles between the fed and fasted conditions [18]. Cistronic analysis of SRC-2 also identifies roles for amino acid metabolism in the liver and loss of *SRC-2* confers disruption of the circadian response in alanine, aspartate, and glutamine metabolism [19]. Within the prostate, SRC-2 regulates citrate production through reductive-carboxylation of α -ketoglutarate promoting lipogenesis (unpublished data). In prostate cancer, SRC-2 stimulates glutamine uptake, and then glutamine signaling activates mTORC1 leading to phosphorylation of SRC-2 and coactivation of transcription factors such as sterol regulatory element-binding protein (SREBP) to effect increases in lipogenic transcriptional programs. This enhancement in cellular lipids increases the energy and membrane intermediates that are required for survival of metastatic prostate cells. Amino acid metabolism is also paramount for optimal brain function as some metabolites function as neurotransmitter precursors. Compared to the other SRCs, loss of SRC-3 altered the largest subset of amino acid metabolites in the brain [18]. These findings suggest that SRC-3 may influence the availability and utilization of neurotransmitter precursors and intermediates. However, the metabolic signature of this brain phenotype is, in part, the result of a skeletal muscle defect in *SRC-3*^{-/-} mice that causes systemic hyperammonemia, leading to elevated glutamate levels and global suppression of amino acid signaling [24]. Thus, SRC-1 and SRC-3 regulate opposite ends of amino acid metabolism with SRC-1 modulating the catabolism of tyrosine whereas SRC-3 regulates the anabolism of glutamate/glutamine as possible neurotransmitter precursors. Finally, as a potent circadian regulator, SRC-2 is involved in maintaining the overall rhythmicity of amino acid flux (Figures 2 and 3).

Xenobiotic Metabolism

The SRCs also play roles in drug and xenobiotic metabolism in the liver. These processes are largely regulated by NR-mediated transcription of genes encoding the cytochrome P450 (*CYP450*) class of enzymes [37]. SRC-1 coactivates several NRs involved in CYP450 regulation including liver receptor homolog-1 (LRH-1), constitutive androstane receptor (CAR), steroid and xenobiotic receptor (SXR), and hepatocyte nuclear factor 4 α (HNF4 α). As one specific example, SRC-1 coactivates LRH-1 on the *Cyp7a1* promoter to regulate bile acid synthesis [38]. SRC-2 and SRC-3 can coactivate LRH-1, suggesting functional redundancy in SRC coactivation of LRH-1 [39,40]. SRC-1 and PGC1 α transactivate hepatocyte nuclear factor 4 (HNF4 α) on the *Cyp2c9*, *Cyp1a1*, and *Cyp1a2* promoters to activate these drug metabolizing enzymes [41]. NR agonists increase coactivator recruitment and expression of xenobiotic targets. Phenobarbital, a CAR agonist, increases SRC-1 and

CAR-mediated activation of *Cyp2b1* expression [42]. Mitotane, a drug used to treat adrenocortical carcinoma, is both a SXR and CAR agonist. Recruitment of SRC-1 to the SXR LBD is increased by mitotane treatment to increase *Cyp3a4* expression, which facilitates regulation of glucocorticoid metabolism in the liver and intestine [43].

SRC-3 coactivates CAR and loss of SRC-3 attenuates the hepatic response to 1,4-bis-[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP, a known CAR agonist), thereby decreasing hyperplasia and decreasing acetaminophen toxicity [44]. Loss of either SRC-1 or SRC-2 fail to show these same effects in response to TCPOBOP treatment, indicating that SRC-3 may be the major coactivator involved in TCPOBOP-induced acetaminophen toxicity, with loss of SRC-3 leading to reduced expression of CAR-mediated expression of drug metabolizing targets genes *Gadd45b*, *Cyp2B10*, *Cyp1A2*, and *Cyp3A11*, in the liver [44]. As a result, SRC-3 increases acetaminophen toxicity, but SRC-1 and SRC-2 reduce toxicity in part through NR coactivation and increased *CYP450* target gene expression.

Steroid metabolism

The SRCs are important mediators of steroid metabolism. SRC-1 regulation of the hypothalamic-pituitary-adrenal (HPA) axis is well characterized [45]. *SRC-1*^{-/-} mice are glucocorticoid-resistant and fail to increase glucocorticoid receptor (GR) target gene expression after dexamethasone treatment. Chronic stress increases corticosterone levels in *SRC-1*^{-/-} mice, but their glucocorticoid-induced stress response is blunted [45]. When overexpressed, SRC-1 splice variants differentially act as corepressors for GR on the corticotropin-releasing hormone (CRH) promoter. SRC-1a, is the primary splice variant with corepressor activities [46,47]. Addition of the selective GR agonist, C108297, suppresses CRH in the hypothalamus and selectively recruits SRC-1a [47]. Interestingly, loss of SRC-1 in males shows decreased anxiety-like behaviors in the elevated-plus maze [48]. In addition to the stress response, SRC-1 is expressed highly in anorexigenic pro-opiomelanocortin (POMC) and steroidogenic factor-1 (SF-1) neurons, suggesting that SRC-1 may regulate feeding behaviors [49]. Indeed, loss of SRC-1 decreases estrogenic-mediated anorexigenic effects and increases weight gain and food consumption [49].

SRC-2^{-/-} mice have structural defects in the adrenal glands, which result from deficits in steroidogenic enzymes culminating in dysregulation of the HPA axis. The resulting HPA hyperactivity is driven by hypo-function of the adrenal glands and lower corticosterone levels [50]. Behavioral analysis of *SRC-2*^{-/-} females, but not males, showed dampened anxiety responses suggesting that SRC-2 dimorphically regulates stress responses; the adrenal gland defect is not wholly responsible for the *SRC-2*^{-/-} stress response [48]. Corticosteroids are important in GR mediated anti-inflammatory responses.

Like SRC-1, SRC-2 functions as either a coactivator or a corepressor for GR [51]. When SRC-2 is recruited directly to the p65 subunit of NFκB and AP-1, it acts as a corepressor for GR and decreases gene expression in the inflammatory response [51]. Macrophages exposed to lipopolysaccharide (LPS) reveal decreased repression of GR and NFκB targets upon *SRC-2* ablation [52]. Interestingly, cistromic analysis of dexamethasone- or LPS-treated macrophages show equal induction of SRC-2 coactivator and corepressor sites [53]. The

interplay of SRC-2 as both a coactivator and a corepressor for GR mediates both inflammatory and metabolic signaling [53].

SRC-3 coactivates progesterone receptor (PR) in pituitary cells and this interaction is increased by gonadotropin releasing hormone (GNRH) or progesterone treatment [54]. Loss of SRC-3 in females increases anxiety responses suggesting a dynamic interplay between the SRCs and the regulation of stress responses [48]. SRC-3 expression in the brain changes as a function of age, being higher in young adult mice (i.e. 25 week old) compared to older male mice (i.e. 70 week old). Likewise, SRC-3 expression decreases in pluripotent cells after differentiation [55,56]. Each of the SRCs play prominent roles in the HPA axis, with SRC-1 regulating CRH production and feeding behaviors in the hypothalamus, SRC-3 being a PR coactivator for gonadotropin α -subunit promoter, and within the adrenal glands, and SRC-2 a major regulator of corticosterone synthesis (Figure 2).

SRC-1 and SRC-2 also have important regulatory roles in cholesterol-mediated signaling via the LXR/RXR heterodimer, which is important not only in lipid and xenobiotic metabolism, but also in cholesterol regulation [57]. SRC-2 coactivation of LXR/RXR is critical for cholesterol biosynthesis in Sertoli cells of the testis and loss of SRC-2 leads to accumulation of cholesteryl esters, which may explain the hypofertility in aging males [58]. Inflammation and metabolism are inherently linked with many NR regulating enzymes serving as both metabolic and inflammatory markers. SRC-1, along with other coactivators and NRs, contributes to cytokine-induced hepatic metabolic changes during the acute phase response (APR) [59]. SRC-1 expression decreases during the APR [60]. Inflammatory-induced APR alters metabolism with IL-1 or TNF treatment decreasing LXR, RXR, PGC1- α , and SRC-2 expression resulting in blunted LXR-mediated APR [61]. Additionally, a number of cholesterol intermediates affect SRC-1 and SRC-2 binding to NRs. Both SRC-1 and SRC-2 show preferential recruitment to LRH-1 upon medium chain phospholipid binding, but are inhibited by long-chain phospholipids [62]. In steroid biosynthesis, protein inhibitor of activated STAT-gamma (PIAS γ) can inhibit SRC-1 binding to LRH-1 on the *Cyp11a1* promoter through competitive binding, thereby decreasing steroid production [38]. Similarly, binding of 24S-hydroxycholesterol inhibits SRC-2 complex formation with ROR α and ROR γ decreasing *Bmal1* and *Rev-erba* expression, molecular components of the mammalian circadian clock [63]. Thus, both SRC-1 and SRC-2 regulate cholesterol synthesis through coordination of LXR/RXR and LRH-1 driven gene transcription.

Concluding Remarks and Future Perspectives

This review highlights the overarching involvement of SRCs in lipid, carbohydrate, amino acid, xenobiotic, and steroid metabolism (Figures 2–3). The SRCs serve as metabolic sensors and coordinators across tissues regulating inputs for diverse processes including, but not limited to, feeding/sleeping behavior, stress response, and reproduction demonstrating that each SRC family member is a conserved master regulator in systems physiology. Coordination of SRC activity within or between tissues is likely to be the result of specific signaling inputs that modulate SRC activities in accordance with metabolic demand. The reported effects of SRC activity on the metabolite landscape provide an elegant, yet effective, method for fine-tuning metabolic transcriptional programs to acutely respond to

the ever-changing energy landscape of the organism. In this manner, the SRCs function at the interface of metabolic gene programs and pathways. SRCs couple the amplitude and timing of metabolic gene expression with the energetic demands of the cell.

Evidence supporting these coordinated regulatory capacities is witnessed by the complex array of interrelated metabolic functions controlled by SRC-2. We have discussed the role of SRC-2 in the liver for promoting dietary lipid uptake while simultaneously managing gene programs for the transport and utilization of fatty acids [20]. Likewise, SRC-2 performs complementary functions in adipose and skeletal muscle that contribute to its role in maintenance of whole body energy homeostasis [12,23]. This concept of integrated regulation is further supported by the recent report that SRC-2 is an essential integrator for circadian rhythm and metabolism through synchronization of tissue selective gene programming [19]. Coupled with these observations, and the fact that SRC-2 directs metabolic programs in the testes and uterus that influence reproductive efficiency, raises the intriguing hypothesis that SRC-2 has evolved as a “master” metabolic integrator. Evidence substantiating this notion of evolutionary metabolic selection stems from the International HapMap Project, which rank and filed the positive selective pressures for all alleles based on different ethnic populations [64,65]. These calculations position coregulators at the top of the list suggesting that genes such as SRC-2 have remained under stringent selective pressure, thus highlighting their importance for the ever-changing metabolic adaptations required for human survival and reproduction.

A key remaining question is how the SRCs can serve as such diverse, yet interconnected regulators of systems metabolism? We envision that PTMs tune these functions of the SRCs. The fact that all PTMs are dissipative energetic events and represent metabolic intermediates or byproducts yields a cycle whereby PTM substrates signal for activation of reparative metabolic pathways to restore PTM reserves. For example, the activation of energy accretion/production pathways via AMPK is driven by ATP consumption and production of AMP/ADP [66,67]. The activation of AMPK enhances SRC-2 transcriptional activity to drive *BSEP* gene expression to promote energy accretion [20]. In the case of SRC-1, both its activity and stability are responsive to fluctuations in glucose levels leading to the selective expression of complex I components of the mitochondrial electron transport chain, which facilitates the conversion of NADH to NAD⁺ [27]. The elevated NAD⁺ pool, in turn, enhances SRC-1 stability to improve the glucose-deprived cellular state. SRC-3 loss-of-function mutations on key phosphorylation sites highlight the importance of PTMs in reprogramming systems metabolism [30]. Several PTM sites and functions have been identified for each of the SRCs, but a comprehensive understanding of how metabolic processes influence PTMs of the SRCs in response to cellular demands remains largely incomplete and represents an important area for future experimentation [30].

While the studies discussed here underscore the importance of SRC regulation in core metabolic tissues, there is a deficit in our understanding of how SRCs function in several metabolic tissues including the pancreas and intestine. Moving forward, defining SRC actions in these tissues will be crucial to understanding their overall roles in coordinating systems metabolism. Additionally, available data suggest that expression of SRC family members can be adjusted in response to dietary cues and changes in the metabolic state, but

the mechanistic underpinnings that explain these events remain largely unexplored [13,26,27]. Moreover, few studies focus on tissue-specific sensors or upstream metabolic regulators for SRC activation or repression. Focusing specifically on activation and repression signals that alter SRC expression/activity will likely facilitate our understanding of signaling between SRCs and their coordinated metabolic functions [68,69].

Glossary

Adaptive thermogenesis	A metabolic response activated in BAT in response to cold exposure that results in uncoupling of mitochondrial respiration for the production of ATP allowing for energy to be dissipated as heat.
Brown adipose tissue (BAT)	a mitochondria rich form of adipose that is responsible for uncoupling mitochondrial respiration for heat generation
High fat diet (HFD)	a diet with a disproportional percentage (i.e. 45 – 60%) of calories from fat. HFD is used to generate diet-induced obesity (DIO) in mouse models to study the effects of metabolic stress such as type 2 diabetes, hypertension, hypercholesterolemia and atherosclerosis.
Hypothalamic-pituitary-adrenal axis (HPA)	an interconnected hormonal signaling network comprised of three endocrine glands (i.e. hypothalamus, pituitary gland and adrenal glands). Signaling through this axis accounts for major hormonal inputs in response to stress and metabolic events such as digestion, energy expenditure and storage.
Ligand binding domain (LBD)	a tightly conserved region of the NR superfamily that confers specificity for ligand binding. The structural confirmation of this domain dictates the NR response to agonists (ligands that activate NR function) or antagonists (ligands/compounds that inhibit NR function).
LxxLL Motif	A contiguous sequence of 5 amino acids where L = leucine and x = any amino acid. The LxxLL domain is also referred to as nuclear receptor (NR) box. The LxxLL domain confers interaction of coregulators to NRs by binding to a groove on the surface of the ligand binding domain of NRs.
Nuclear receptor (NR)	a 48-member class of DNA binding transcription factors (TF). The superfamily is comprised of four classes of NRs: 1) Those that homodimerize (i.e. GR, ER, PR, AR); 2) Those that heterodimerize with RXR (i.e. RAR, PPAR, VDR, TR); 3) Those for which a native ligand has been identified (i.e. estrogen for ER); 4) Orphan receptors for which no ligand has been defined.
Oxidative phosphorylation	The metabolic process executed in the mitochondria by which ATP is generated from successive series of electron transport or

	<p>redox reactions. These redox reactions require the use of the coenzyme NADH (the protonated form of nicotinamide adenine dinucleotide), which is produced by various catabolic biochemical processes (i.e. glycolysis, TCA cycle, β-oxidation). NADH is consumed during oxidative phosphorylation to form NAD^+.</p>
Peroxisome proliferator-activated receptor gamma coactivator 1 α/β (PGC1α/β)	<p>a potent transcriptional coactivator (for PPAR & CREB) that drives the activation of gene expression programs for mitochondrial biogenesis, fatty acid metabolism and glucose homeostasis.</p>
Post-translational modification (PTM)	<p>the dissipative process by which biochemical substrates are covalently added to accessible amino acid residues of proteins. The majority of PTMs require enzymatic machinery to catalyze the addition of the chemical moiety to the target protein. PTMs influence target protein localization, activity, stability and complex constituency.</p>
Steroid Receptor Coactivators (SRCs)	<p>a structurally related three-member family of p160 transcriptional coregulators whose classical functions are to serve as amplifiers for gene transcription.</p>
Tricarboxylic acid cycle (TCA Cycle)	<p>also known as citric acid cycle, a series of inter-dependent chemical reactions from which ATP is produced from the oxidation of acetate.</p>
Uncoupling protein 1/3 (UCP1/3)	<p>proteins localized in the inner mitochondrial membrane which function to dissipate the proton gradient to uncouple ATP production for heat production.</p>
White adipose tissue (WAT)	<p>a storage cell type for deposition of excess fat. WAT also provides an insulator function. Several WAT-derived hormones are produced, referred to as adipokines, which signal to peripheral tissues (i.e. liver, brain) to influence whole body energetic programs.</p>

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Box 1: Historical Perspective of Coregulators

Our laboratory began studying NR-interacting proteins in the 1970s after detecting PR-associated protein complexes bound to DNA [70]. Our extensive efforts to purify what we considered to be a few “acceptor proteins” were largely unsuccessful due to their ‘mystifying’ size and charge distributions across multiple separation technologies. We would later learn that our attempts were unsuccessful because coactivators actually were not one of a few proteins, but were composed of hundreds of different “acceptor proteins.” Two decades later, and in accord with these findings, Murray and Towle published thyroid hormone receptor (TR)-associated proteins that changed in the presence and absence of ligand [71]. Around the same time, Ma and Ptashne showed in yeast cells that yeast protein interactions with TFs can alter gene expression [72]. In the 1990s, a surge of papers from Tjian and colleagues coined the term “coactivator” for proteins they identified in *Drosophila* that enhanced TATA Binding Protein activity at promoters [73]. In 1992, the Roeder lab published results indicating that these “acceptor proteins” could enhance gene activity [74]. Also in 1992, our laboratory gleaned more definitive molecular understanding of NR repression through elucidating a NR-repressor domain within the carboxyl-terminus of PR and RAR that could be altered in the presence of agonists and antagonists [76,77]. Later in 1992, we published the first proof-of-principle study showing that NR function could be directly influenced by a coregulator [75]. Later, Goodman showed that cAMP response element-binding protein (CREB)-binding protein (CBP) could enhance CREB-mediated transcription [78]. In 1994, Brown identified estrogen-interacting protein fractions termed “ERAP160s” that bound ligand-activated ER [79]. Similarly, we identified SPT6 (TAF2) as a functional coactivator for ER [80]. Our laboratory and others including the Glass, Chen and Evans laboratories definitively showed that corepressor exchange for a coactivator enhanced NR activity [80–82]. In 1995, our laboratory was finally successful in cloning the first authentic NR coactivator (SRC-1) and began characterizing its *in vivo* function in mice and the physiological importance of the SRC family of coactivators (SRC-1, SRC-2 and SRC-3) [84]. These findings provided the first definition of coregulators as proteins that bind directly to NRs and other TFs, but not directly to DNA. NRs like other TFs bind to specific response elements in the genome and subsequently recruit coregulators. In general, recruitment of coactivators enhances gene transcription whereas recruitment of coregulators represses gene transcription. Additionally, NR activity can be modulated using either agonists that promote binding of coregulators to NRs or antagonists that inhibit binding to NRs [83]. To date, over 450 coregulators have been identified with thousands more co-coregulators identified that participate in complexes with ‘primary’ coactivators [85]. Despite this overwhelming number of coregulators, we remained focused on one structurally related family of coregulators (i.e. the SRCs) that were involved in the activation of gene transcription to explore their mechanistic and physiological functions. While previously thought of as just “acceptor proteins”, we can now appreciate that coactivators interact in large protein complexes with other chromatin modifiers, co-coregulators, and TFs as further validated in high-throughput mass spectrometric analysis [85]. For nearly two decades now the field of coregulator research continues to highlight the amazing potential of these molecules to impact physiological

outcomes. Here, we provide a comprehensive, yet focused, summary of the molecular contributions of the SRCs to metabolic systems physiology.

Box 2: Outstanding Questions

- Which are the signaling activators (metabolites, hormones, etc.) that direct SRC function?
- What are the metabolic tissue- or cell-specific chromatin interactions of the SRCs?
- What are the metabolic functions of the SRCs in currently understudied tissues (i.e. pancreas and brain)?
- How do the SRCs coordinately regulate metabolic stress responses (i.e. food entrainment, caloric restriction)?
- How is expression of the SRC genes (i.e. Ncoa1, Ncoa2, Ncoa3) controlled by metabolic disruption/stress and diverse signaling pathways?
- How are SRC protein dynamics (i.e. turnover, stability) influenced by metabolic disruption/stress?
- What are additional non-NR SRC functions?
- What is the totality of functions of the SRCs in cancer cell metabolism?

Highlights

- SRCs are required for diverse metabolic processes
- SRCs impact metabolic systems physiology
- SRC dysfunction confers metabolic pathophysiology
- SRCs bridge anabolic and catabolic functions for maintenance of energy homeostasis

Figure 1. Systems biology summary of SRC tissue-specific metabolic functions

Process summary of SRC functions in core metabolic tissues (brown adipose tissue (BAT), brain, liver, heart, adrenal gland, intestine, muscle and white adipose tissue (WAT)). Within the brain, SRC-1 regulates CRH expression and feeding behaviors, SRC-2 is a major regulator of circadian rhythm, and SRC-3 regulates neurotransmitter flux by altering glutamate/glutamine metabolism. In BAT, SRC-1 promotes energy expenditure through increasing thermogenesis, and SRC-2 and SRC-3 suppress thermogenesis but also promote adipogenic gene programs. In liver, SRC-1 regulates euglycemia and amino acid metabolism. SRC-2 is a regulator of fasting glycemia, diurnal metabolism, and dietary fat

absorption. SRC-3 promotes lipolysis in the liver. Within the heart, SRC-2 is a coactivator involved in cardiac hypertrophy and SRC-3 maintains cardiac rhythmicity. SRC-2 increases fat absorption in the intestine and in the adrenal glands promotes steroidogenesis. In skeletal muscle, SRC-1 promotes energy expenditure whereas SRC-2 suppresses energy utilization. SRC-3 regulates fatty acid fuel usage to drive muscle endurance. In WAT, SRC-1 promotes energy consumption with SRC-2 oppositely regulating energy storage. As with BAT, SRC-2 and SRC-3 promote adipogenesis.

Figure 2. Summary of SRC pathway-specific metabolic functions

Summary of SRC functions in lipid, carbohydrate, amino acid, xenobiotic, and steroid metabolic processes. The illustration shows a summary of the overarching roles for the SRCs in these metabolic processes, with the specific mechanisms listed to the side. In lipid metabolism, SRC-1 promotes lipid utilization through increased stability of PGC1α/PPARγ and decreased β-oxidation, reduced fatty acid oxidation and mitochondrial oxidative phosphorylation. SRC-2 and SRC-3 promote lipid storage. SRC-2 decreases the stability of PGC1α/PPARγ, increases *BSEP* transcription through FXR, and increases oxidative

phosphorylation. SRC-3 increases lipid storage through decreased *GCN5* expression to promote PGC1 α action and increased fatty acid metabolism in muscle. Each of the SRCs are involved in carbohydrate utilization with SRC-1 regulating pyruvate metabolism, and a subsequent increase in *SRC-1* expression during fasting. SRC-2 regulates fasting glucose and glycolytic flux through increased *G6PC* expression via ROR α . SRC-3 regulates insulin signaling through increased *IGFBP3* expression. In amino acid metabolism, SRC-1 and SRC-3 regulates utilization while SRC-2 controls diurnal rhythm of amino acid metabolites. SRC-1 is a regulator of tyrosine through increased TAT expression and SRC-3 increases glutamate/glutamine metabolism. SRC-1 and SRC-2 have protective roles in drug/xenobiotic metabolism with increased expression of CYP450 enzymes via NR coactivation. SRC-3 increases acetaminophen toxicity through CAR coactivation. In steroid metabolism, SRC-1 is a hypothalamic regulator of CRH expression and ER-mediated feeding behaviors. SRC-2 regulates the development of the adrenal glands and steroidogenesis of corticosterone. SRC-2 is also an important mediator of cholesterol synthesis. Within the pituitary gland, SRC-3 increases PR target gene expression with the GNRH ligand.

Figure 3. Anabolic versus catabolic functions of the SRCs

Schematic representing the anabolic versus catabolic processes impacted by SRC-1 (blue), SRC-2 (red) and SRC-3 (green) for amino acid, carbohydrate and lipid metabolism. Size of the SRC denotes its relative importance to that pathway's regulation and overlapping SRCs signify dual control. Within carbohydrate metabolism, SRC-1 and SRC-3 predominantly regulate anabolic processes including gluconeogenesis and glucose uptake by promoting insulin signaling. SRC-2 is involved in glucose accessibility through catabolism of glycogen. In lipid/fat metabolism, SRC-2 is involved in both catabolic and anabolic

processes including bile acid metabolism, adipogenesis, and lipid storage in adipose. SRC-1 regulates anabolic lipid and fat metabolism, and SRC-3 regulates long chain fatty acid catabolism. SRC-3 promotes anabolic gene programs in amino acid metabolism, and SRC-1 promotes catabolic processing of amino acid metabolism especially through tyrosine. As a diurnal regulator, SRC-2 maintains equal flux of anabolic and catabolic gene programs.