

Recent progress in the biology and physiology of sirtuins

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The sirtuins are a highly conserved family of NAD⁺-dependent enzymes that regulate lifespan in lower organisms. Recently, the mammalian sirtuins have been connected to an ever widening circle of activities that encompass cellular stress resistance, genomic stability, tumorigenesis and energy metabolism. Here we review the recent progress in sirtuin biology, the role these proteins have in various age-related diseases and the tantalizing notion that the activity of this family of enzymes somehow regulates how long we live.

"Chekhov's gun" refers to the seemingly irrelevant prop or observation that is introduced early on in a drama (such as the gun in Act I of *Uncle Vanya*) but whose true significance is only revealed at the play's conclusion. Often, science has its own version of the Russian playwright's famous maxim. In our case, the drama begins in the autumn of 1914, with the seemingly irrelevant observations of a young professor describing the beneficial effects of rats placed on a calorie-restricted diet. Some suspect that these remarkable observations were largely ignored and forgotten because of concerns about the scientist himself. Indeed the young professor, F. Peyton Rous, had only a few years earlier shocked the scientific community by postulating that in some situations, cancer could be transmitted by viruses—a notion that was roundly ridiculed and derided by his older colleagues. For whatever reason, Rous's curious observations remained dormant for nearly two decades. Over time, however, other scientists gradually extended these ideas and demonstrated the benefits of caloric restriction in species ranging from humans to yeast. Indeed, it was in the latter species that dissection of the molecular basis of caloric restriction first implicated a family of NAD⁺-dependent enzymes, now collectively termed the sirtuins. In the following sections, we review the recent progress in our understanding of sirtuin biology with particular emphasis on their role in mammalian species. Although much remains unanswered and controversies remain unresolved, as with Chekhov's gun it is only with the passage of time that the true significance of Rous's observations can finally be understood.

A new family of deacetylases

The founding member of the sirtuin family, yeast Sir2 (silent information regulator 2), was originally isolated in a screen for silencing factors¹. Ultimately, four proteins (named Sir1–Sir4) were established as important regulators of silencing at the mating type locus as well as telomeric DNA. The Sir family was rescued from relative obscurity when Guarente and colleagues, using *Saccharomyces cerevisiae* as a model system, independently identified these proteins as key regulators of lifespan^{2,3}. Subsequent analysis revealed that Sir2 functioned biochemically as a histone deacetylase, in a unique reaction requiring the energetic intermediate NAD⁺ as a co-factor⁴. Remarkably, analysis in other model organisms (including *Caenorhabditis elegans* and *Drosophila*) also implicated Sir2 homologues as a determinant of lifespan⁵. In both yeast and flies, Sir2 levels increased following caloric-restriction treatment. Perhaps more

importantly, in sirtuin-deficient yeast and mice, extension of lifespan by caloric restriction is abolished^{5,6}. Subsequent studies have qualified some of these observations and have demonstrated that in yeast, the effect of Sir2 in caloric restriction and lifespan extension might be strain-, species- and context-dependent^{7,8}.

To date, seven mammalian homologues have been identified, with mammalian SIRT1 closest evolutionarily to yeast Sir2. Specific mouse models in which one or more sirtuin genes has been knocked out have been recently created (Fig. 1). Cell biological studies have further demonstrated different subcellular compartments for each family member, with SIRT6 and SIRT7 being nuclear proteins, SIRT3, SIRT4 and SIRT5 mitochondrial proteins, and SIRT1 and SIRT2 being found both in the nucleus and the cytoplasm, in a cell- and tissue-dependent context⁹. The observation that these proteins

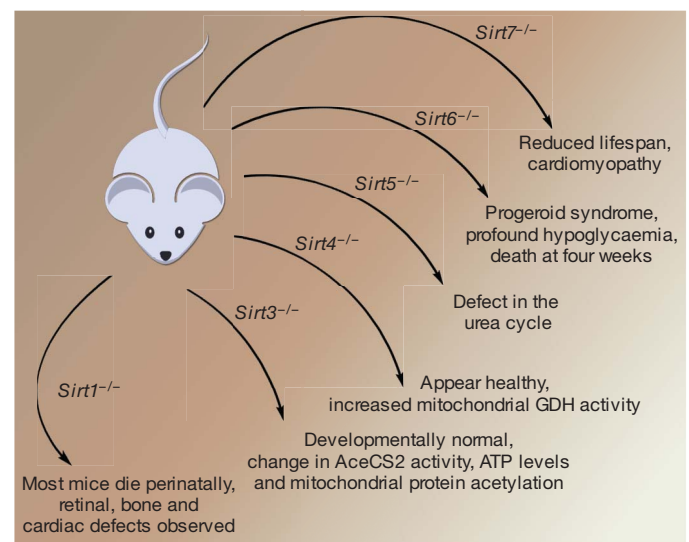


Figure 1 | Mouse knockout models as tools for exploring sirtuin function. Gene targeting of SIRT1, SIRT3, SIRT4, SIRT5, SIRT6 and SIRT7 has been reported. The phenotypes include a reduction in median lifespan, ranging from a usual survival of days (SIRT1) to weeks (SIRT6) or months (SIRT7). In contrast, although biochemical phenotypes have been reported, *Sirt3*^{-/-} and *Sirt4*^{-/-} mice appear outwardly normal. Initial reports suggest that *Sirt5*^{-/-} mice also exhibit no obvious phenotype⁴⁹.

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require NAD^+ as part of their enzymatic action immediately suggested a mechanistic link between sirtuin activity and intracellular energetics. However, it is important to stress that currently there is no definitive evidence that these proteins play any direct role in mammalian lifespan regulation, as neither pharmacological sirtuin activators nor overexpression of SIRT1 has been demonstrated to extend lifespan in mice. Nonetheless, the explosion of interest in the sirtuins centres on the nagging notion that in some fashion, these proteins provide the connection between what we eat and how long we live.

Sirtuins in DNA repair

A role for sirtuins in maintaining genomic integrity was suggested by early work in model organisms. For instance, yeast Sir2 was shown to inhibit recombination of ribosomal DNA as well as to relocalize to sites of DNA breaks^{5,10}. Furthermore, yeast sirtuin family members are capable of deacetylating histone proteins and the absence of this activity results in silencing defects, increased genomic instability and sensitivity to DNA damage^{5,10}. These properties appear to also extend to the mammalian sirtuins. The first example came with analysis of SIRT6-deficient cells that demonstrated increased sensitivity to genotoxic damage and accumulation of chromosomal abnormalities¹¹. Recent studies have shown that SIRT6 also acts as a histone deacetylase that can influence the telomeres of human cells¹².

Mammalian SIRT1 also appears to influence genomic stability. First, SIRT1 can deacetylate various factors linked to the repair of DNA damage, including the Werner helicase and NBS1 (ref. 13). A role for SIRT1 in genomic integrity was further substantiated by the recent demonstration of increased chromosomal aberrations and impaired DNA repair in *Sirt1*^{-/-} embryos¹⁴. In addition, following oxidative damage, SIRT1 has been shown to be recruited to sites of DNA breaks. Although such recruitment appears to be important in protecting against genomic instability, this response is accompanied by the de-repression of previously silenced genes¹⁵. These observations suggest that like yeast Sir2, SIRT1 appears to regulate epigenetic silencing and chromatin modification, and recent evidence suggests this is achieved, at least in part, through direct regulation of modifying enzymes, such as the histone methyltransferase SUV39H1 (refs 16, 17).

Sirtuins and cell fate

Mammalian sirtuins also appear to have an important role in regulating cellular stress resistance and modulating the threshold for cell death. In part, this increased stress resistance comes from the interaction with the Forkhead box class O (FOXO) family of transcription factors. These mammalian transcription factors regulate both energy status and stress resistance, two properties intimately connected to lifespan extension. SIRT1 can bind and deacetylate FOXO3a, leading to a selective augmentation of FOXO-regulated stress resistance genes^{18,19}. Subsequent experiments have extended these observations to demonstrate that various different sirtuin and FOXO family members can interact. In addition, a recent report suggested that SIRT1 can also protect cells against stress by regulating the heat shock response²⁰. The interaction of SIRT1 and p53 can also modulate the threshold for cell death in the setting of exogenous stress^{21,22}. Indeed, FOXO proteins and p53 proteins can directly interact under stress conditions^{18,23}, and p53 can regulate SIRT1 expression through various means that include p53-dependent microRNAs²³⁻²⁵. Besides p53, SIRT1 can regulate other targets linked to cell death, including Ku70, E2F1 and TGF- β signalling²⁶. In addition, the coordinated action of SIRT3 and SIRT4 appear to inhibit cell death by maintaining mitochondrial NAD^+ levels following stress²⁷. Although in most examples sirtuin activity appears to antagonize stress-induced cell death pathways, SIRT1 can also deacetylate components of the NF- κ B complex, leading to increased apoptosis²⁸.

Given the potential link between sirtuins and ageing, it is appealing to envisage a role for these proteins in mediating processes such as cellular senescence and stem cell function. Consistent with such a role, SIRT1 expression can antagonize the premature senescence seen

in mouse embryonic fibroblasts following forced oncogene expression²⁹. Similarly, through its regulation of NF- κ B activity, knockdown of SIRT6 appears to augment senescence in primary keratinocytes³⁰. However satisfying it may be to conclude that increased sirtuin activity can forestall senescence, it is important to note that an analysis of studies of *Sirt1*^{-/-} mouse embryonic fibroblasts has come to the opposite conclusion³¹. With regards to differentiation, sirtuins have been shown to inhibit adipogenesis by modulating PPAR- γ (peroxisome proliferative activated receptor γ) and to effect muscle and neuronal differentiation^{32,33}. Recent studies in mouse embryonic stem cells also suggest a role for SIRT1 in regulating stem cell reactive oxygen species homeostasis and differentiation³⁴. The observation that SIRT1 can also interact with members of the polycomb family of repressors, a family of proteins intricately connected to stem cell self-renewal, suggests that additional links between sirtuins, differentiation and stem cell function are likely to emerge³⁵.

Sirtuins and metabolic regulation

In mammals, blood glucose concentration is maintained within a narrow range under a variety of physiological conditions. During starvation, maintenance of serum glucose is achieved in part by implementing a program of hepatic gluconeogenesis. Increasing evidence suggests an important role for sirtuins in this physiological adaptation. The peroxisome proliferator-activated receptor gamma-coactivator-1 α (PGC-1 α) is a known target of SIRT1-dependent deacetylation^{36,37}, and this coactivator also plays a fundamental part in regulating gluconeogenesis and fatty acid oxidation pathways within the liver. The ability of PGC-1 α to modulate these latter two pathways appears to require SIRT1 (refs 37, 38). Recently, distinct roles for protein acetylation and SIRT1-dependent deacetylation have been shown to regulate the hepatic response to both short term (<6 h) and long term (>18 h) fasting³⁹. In this case, the opposing actions of SIRT1 and the p300/CBP acetyltransferase choreograph hepatic glucose production in the setting of nutrient stress. Finally, the observation that SIRT6-deficient mice demonstrate severe hypoglycaemia suggests a potential role for other sirtuins in glucose production and homeostasis¹¹.

In addition to regulating hepatic gluconeogenesis, sirtuins also modulate serum glucose levels by regulating pancreatic insulin secretion. A transgenic mouse with β cell-specific SIRT1-overexpression was noted to have increased glucose-stimulated insulin secretion and improved glucose tolerance compared to control mice⁴⁰, while *Sirt1*^{-/-} mice have impaired glucose-stimulated insulin secretion⁴¹. Finally, SIRT4 through its ADP-ribosylation of glutamate dehydrogenase (GDH)⁴² as well as its interactions with insulin degrading enzyme⁴³ also appears to be involved in regulating pancreatic insulin secretion.

The sirtuin family has a much broader role in metabolism than the regulation of glucose homeostasis (Fig. 2). As was previously discussed, SIRT1 through its regulation of PPAR- γ and PGC-1 α activity has a significant regulatory role in fat mobilization and fatty acid oxidation^{32,44}. The regulation of PGC-1 α activity also suggests a role for sirtuins in the generation of new mitochondria, as PGC-1 α is a key regulator of mitochondrial biogenesis. These observations, coupled with recent links between SIRT1 and autophagy⁴⁵, suggest that sirtuins might regulate the flux of mitochondria within cells by balancing PGC-1 α mediated generation with autophagy-dependent clearance. In addition to the connection between SIRT1 and the mitochondria, SIRT3-dependent deacetylation regulates the activity of the mitochondrial enzyme acetyl coenzyme A synthetase 2 (AceCS2)^{46,47} as well as Complex I of the electron transport chain⁴⁸. Mitochondrial lysates from *Sirt3*^{-/-} mice reveal >30 proteins whose acetylation is markedly increased, suggesting that other important targets undoubtedly exist^{48,49}. Finally, a very recent study has implicated SIRT5, another mitochondrial sirtuin, as an important regulator of the urea cycle⁵⁰.

Although the role of sirtuins in regulating metabolism has centred on key metabolic organs, such as liver and pancreas, early studies in

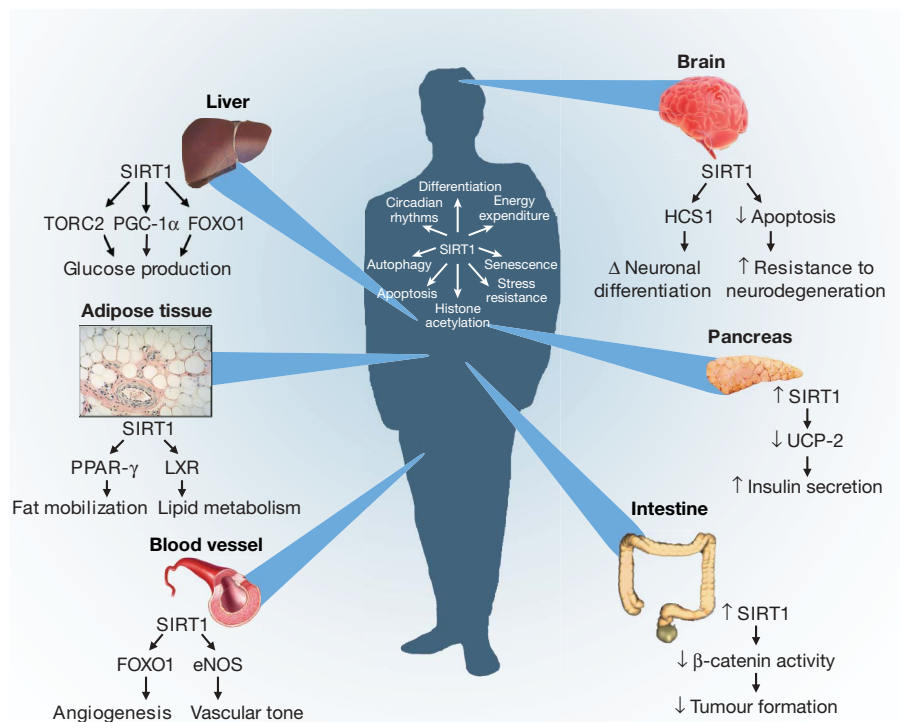


Figure 2 | The diverse physiological roles of the sirtuins. Shown are examples of the organ-specific physiology of SIRT1, along with some of the direct or indirect targets of sirtuin regulation (see text for details). In addition, examples of some of the SIRT1-regulated intracellular parameters are presented, ranging from modulation of progenitor differentiation to altering the threshold for apoptosis. At the beginning of a compound name or process (for example, 'Tumour formation' at bottom right), down-arrow indicates 'decreasing', up-arrow indicates 'increasing' and 'Δ' indicates 'change in'.

human subjects undergoing voluntary caloric restriction suggest that levels of SIRT1 rise in tissues as diverse as skeletal muscle and circulating mononuclear cells^{51,52}. The role of sirtuins in the metabolic adaptation of these cell types is largely unexplored. The intriguing connection between SIRT1 and circadian rhythms provides a glimpse of the wide spectrum of metabolic effects sirtuins may eventually be shown to regulate^{53,54}. The observation that SIRT1 can directly deacetylate core components of the circadian clock machinery is particularly fascinating, as the ultimate goal of such rhythms is to coordinate the sleep-wake cycle of an organism with environmental cues, including coordinating and matching intracellular metabolism to external food availability.

Sirtuins and age-related diseases

Given the wealth of data connecting sirtuins to glucose homeostasis and insulin secretion, it seemed reasonable to suspect that these proteins might also regulate the susceptibility to developing insulin resistance and diabetes. Two recent studies using moderate transgenic overexpression of SIRT1 demonstrate that such engineered animals do indeed exhibit improved glucose tolerance when challenged with high-fat diets^{55,56}. Interestingly, these two studies came to two different conclusions regarding the effects of moderately increased SIRT1 expression on basal energy homeostasis. In addition to these gain of function studies, inhibition of SIRT1 by genetic or pharmacological means can induce insulin resistance⁵⁷. These data support the notion that manipulating sirtuin activity might result in protection from a host of metabolic derangements. Such notions have also been aided by the association of SIRT1 genetic variants with human energy expenditure and obesity^{58,59}. In animal models, treatment with the sirtuin activator resveratrol appears to protect against diet-induced obesity and glucose intolerance^{60,61}. Some concerns have been raised regarding whether resveratrol in mammalian systems works exclusively or even predominantly through regulation of sirtuin activity. For instance, resveratrol also activates the AMPK pathway, although recent evidence suggests that AMPK activity is intimately connected to sirtuin function⁶². Some of the concerns raised regarding the specificity of resveratrol have also been addressed by the use of newer and seemingly more specific sirtuin activators, which again appear to provide some protection in animal models of diet-induced obesity and insulin resistance^{63,64}.

Although the link between sirtuins and metabolic disease has been intensely studied, there is a growing body of evidence that similar relationships may hold for a wide swath of age-related maladies. For instance, the ability of SIRT1 to regulate p53 activity potentially implicates the sirtuins in tumorigenesis. Indeed, depending on the context, arguments have been made that SIRT1 might either increase or decrease cancer risks⁶⁵. Recently, the notion that SIRT1 might act as a non-traditional tumour suppressor was highlighted by observations that in the context of a *p53*^{+/-} mouse, haploinsufficiency of SIRT1 resulted in increased tumour formation¹⁴ while overexpressed SIRT1 reduced tumours¹⁵. In addition, a direct connection between the tumour suppressor Brca1 and SIRT1 expression has recently been established⁶⁶. Finally, overexpression of SIRT1 can inhibit tumorigenesis in a mouse model of colon cancer triggered by constitutive Wnt signalling⁶⁷. With observations that SIRT6 regulates DNA repair, and that SIRT2 activity is necessary for proper cytokinesis, a strong possibility exists that other sirtuin family members will also affect cancer predisposition.

The sirtuins also appear to have a prominent role in vascular biology, and may regulate aspects of age-dependent atherosclerosis. Part of these effects may come through regulation of lipid and cholesterol metabolism, including the ability of SIRT1 to modulate the activity of the nuclear receptor LXR, a critical factor in reverse cholesterol transport⁶⁸. In addition, a conditional deletion of SIRT1 in endothelial cells has been demonstrated to impair the angiogenic response following an ischaemic insult⁶⁹. SIRT1 can also deacetylate and regulate endothelial nitric oxide synthase (eNOS) activity, a key regulator of vascular tone⁷⁰. These results are particularly interesting, because in mouse models, caloric restriction has been shown to induce mitochondrial biogenesis through an eNOS-dependent pathway⁷¹. Other sirtuins can also affect cardiovascular physiology, as SIRT7-deficient mice exhibit cardiac abnormalities⁷² and the important vasoconstrictor angiotensin II regulates SIRT3 expression⁷³.

A unified picture of the role of sirtuins in neurological diseases has yet to emerge. Initial reports had implicated SIRT1 in a specific model of axonal degeneration⁷⁴, although subsequent studies have raised the possibility that the neuronal pathology observed in this model may occur through a sirtuin-independent pathway⁷⁵. Similarly, although (as discussed above) SIRT1 is in general thought to be protective against oxidative stress, there is evidence both *in vitro*

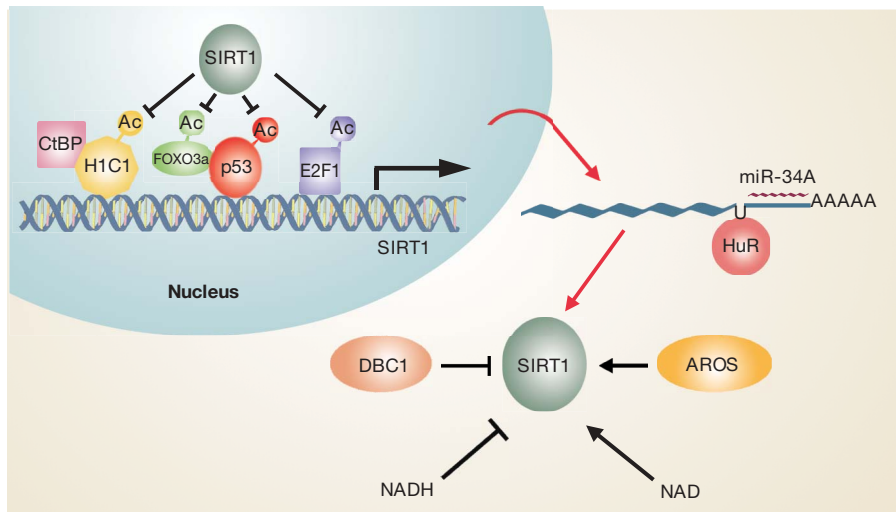


Figure 3 | Complex regulation of SIRT1 activity. The promoter of SIRT1 is positively and negatively regulated by the binding of various transcription factors and repressors, including H1C1, CtBP, p53, FOXO3A and E2F1. The acetylation and hence activity of many of these factors are in turn controlled by SIRT1. The SIRT1 message is also regulated by the RNA binding protein HuR and the p53-regulated microRNA miR-34A. Finally, SIRT1 protein activity is regulated positively and negatively by interacting proteins such as AROS and DBC1, as well as by the overall metabolic state, as reflected in the NAD⁺/NADH ratio.

and *in vivo* that this may not hold true in the brain⁷⁶. Despite these observations, in a mouse model of Alzheimer's disease, brain specific overexpression of SIRT1 appears to reduce neurodegeneration^{77,78}. Surprisingly, pharmacological or genetic inhibition of SIRT2 was also protective in both a cell-based and a *Drosophila* model of Parkinson's disease⁷⁹. These results suggest that increased SIRT1 or decreased SIRT2 both reduce neurodegeneration, potentially raising cautionary flags for the development of broadly acting sirtuin-based pharmaceuticals for these types of conditions.

Concluding remarks

From their humble origins as silencing factors in yeast, members of the sirtuin family have emerged as broad regulators of cellular fate and mammalian physiology. As impressive as the recent progress has been, much of our understanding has come from studies involving only SIRT1, whereas the other six mammalian sirtuins—presumably equally important—have received significantly less attention. Even for SIRT1, many questions remained unanswered, including a full understanding of how the activity of the enzyme is regulated under both normal and stress conditions, as well as during ageing (Fig. 3). The recent discovery of interacting proteins that can both positively and negatively regulate SIRT1 deacetylase activity^{80–82} suggests that similar interacting proteins may exist for the other sirtuin family members. Pharmacological manipulation of sirtuin activity that began with the use of resveratrol has now extended to a variety of newer agents that appear to have greater specificity. Some of these agents have already begun human clinical trials⁸³. Whereas the primary benefit of these drugs may lie in preventing diet-induced metabolic disorders, direct effects on vascular health, tumorigenesis and even overall lifespan are at least within the realm of possibility, given the wide spectrum of sirtuin biology.

From an evolutionary standpoint, it remains unclear why the sirtuin family has emerged as a key regulator of so many seemingly varied processes. Perhaps some of this prominence can be attributed to the enzyme's dependence on NAD⁺, forever linking sirtuin activity to the underlying metabolic state of the cell. In the 1940s and 50s, it became clear that ingested sugar, fat and protein was eventually reduced to a single, simple and versatile intermediate. Fifty years later, it was realized that this same intermediate, acetyl-CoA, could also be used to modify histones and hence regulate gene expression. As such, acetylation and NAD⁺-dependent deacetylation have emerged as perhaps the most immediate and versatile connection between intracellular energetics and intracellular fate. Indeed, the evolving and intricate connection between acetylation, energetics and gene expression is now just beginning to be revealed⁸⁴. In this regard, although numerous individual studies have linked sirtuins to changes in gene expression, regulation of genomic stability or

alterations in intracellular metabolism, the real excitement lies in the understanding that these disparate functions are perhaps all interconnected—that sirtuins serve as the bridge between what we eat and what we are. More twists and turns await us in this drama, as we are indeed far from the final curtain. But for now at least, many of the curious observations of the past are beginning to make sense. After waiting for nearly a century, the significance of Chekhov's gun may have finally been realized.

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