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Bovine TB in livestock and wildlife: what’s in the genes?

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le Roex N, van Helden PD, Koets AP, Hoal EG. Bovine TB in livestock and wildlife: what’s in the genes?. Physiol Genomics 45: 631–637, 2013. First published June 11, 2013; doi:10.1152/physiolgenomics.00061.2013.—Bovine tuberculosis (BTB) is a chronic, infectious disease found in domestic livestock and wildlife. It is caused predominantly by Mycobacterium bovis, which forms part of the Mycobacterium tuberculosis complex. BTB has serious implications for the movement of animals and animal products, biodiversity, and public health and is of significant economic concern. The existence of wildlife maintenance hosts makes it extremely difficult to eradicate BTB, even when established control strategies are in place, creating the need for alternative methods for controlling this disease. There are multiple factors that influence the outcome of infection by a pathogen, one of which is the host’s genome. The identification of genetic variants involved in the susceptibility to BTB would supply a new selection of potential drug targets as well as the possibility for the breeding of animals with greater disease resistance. In this review, we collate the results of the BTB heritability and association studies performed in cattle and wildlife, discuss considerations and other methodologies (such as gene expression work) to be taken into account when performing genetic studies, and make some recommendations for future work in this area.

bovine; tuberculosis; genetics; susceptibility

BOVINE TUBERCULOSIS (BTB) in domestic livestock and wildlife is caused by Mycobacterium bovis, which forms part of the Mycobacterium tuberculosis complex (45). It is a chronic, infectious disease that is transmitted predominantly by inhalation and is characterized by the formation of granulomas in the lungs, lymph nodes, and various other organs (37). The exact origin of this disease is unclear, but it has been suggested that BTB emerged in Europe and spread to the United Kingdom and was subsequently introduced to many parts of the world through the movement of cattle from the UK and the Netherlands to former colonies (38). BTB is widespread in cattle around the world and is present in many wildlife species and domestic animals. This has serious implications for international trade and export of animals and animal products, including beef. Additional economic losses are suffered through the slaughter of livestock, and regular BTB testing incurs a vast expense (32). BTB is also a public health concern, as it is a zoonotic disease and can be transmitted to humans. This is of particular concern in developing countries, where people and livestock live in close proximity to each other (5, 32).

Much interest has recently been focused on the role of wildlife reservoirs in the maintenance and spread of BTB, both within wildlife populations and to domestic livestock and other spill-over hosts. The brushtail possum (Trichosurus vulpecula) in New Zealand, the African buffalo (Syncerus caffer) in South Africa, the Eurasian badger (Meles meles) in the UK, the white-tailed deer (Odocoileus virginianus) in the USA, and the European wild boar (Sus scrofa) in Spain are all considered maintenance hosts of BTB in their respective environments (38). The list of spill-over hosts is far more extensive and includes goats (Capra hircus), Iberian lynx (Lynx pardinus), lions (Panthera leo), dogs (Canis familiaris), cats (Felis catus), sheep (Ovis aries), mink (Lutra lutra), and many more (5, 27).

Multiple factors influence the outcome of infection by a pathogen. Host genetic factors interact with both environmental factors and the genome of the pathogen to determine the disease profile of an individual, with a particular interplay between them resulting in active disease. Many lines of evidence convincingly demonstrate that the host’s genetic make-up plays a role in the outcome of infection with TB in both humans and animals. Twin studies have shown that when monozygotic and dizygotic twins are compared with respect to development of TB, the concordance is higher in monozygotic twins than in dizygotic twins (15). In 1926, 251 babies unintentionally injected with a live dose of virulent M. tuberculosis

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showed a range of responses, demonstrating that there were innate factors involved in the outcome of infection (39). With respect to M. bovis susceptibility, in the 1940s it was found that inbred strains of rabbits displayed one of two phenotypes (susceptible or resistant) following infection with M. bovis. This inheritance pattern suggested that the disease profile displayed by the rabbits was largely genetically inherited (26). The differential susceptibility of mice to the M. bovis Bacille Calmette-Guerin (BCG) vaccine led to the identification of the first TB susceptibility locus, namely the Nramp1 gene (22).

There are many different prevention and treatment strategies used by different countries on different BTB hosts: test and slaughter policies, the BCG vaccine, wide-scale culling and restricted movement, and legislation. All of these strategies have met with limited success in countries where a wildlife BTB reservoir exists, and there is a pressing need for alternative methods of controlling this disease (5, 32). The identification of genetic variants involved in susceptibility to BTB would supply a new selection of potential drug targets as well as the possibility of breeding animals with greater disease resistance. Research into genetic resistance to BTB has begun in a number of countries (Fig. 1), and the application of genetic resistance information to breeding programs has already been implemented in cattle for other diseases such as mastitis (43). Genetic and immunological information form an essential part of this approach, and breeding strategies for BTB resistance, either by marker-assisted selection or the incorporation of marker information into estimated breeding values for a whole-genomic selection approach (31), could be invaluable for both the commercial and private sectors. No single study design or approach can be relied on to identify all the genetic components of TB susceptibility, as each different approach has its own advantages and limitations. Heritability and association studies are the most commonly used approaches in investigating the genetic basis of disease susceptibility and will be the focus of this review.

Below, we summarize the results of the BTB heritability and association studies performed in cattle and wildlife, suggest considerations and other methodologies to be taken into account, and make some recommendations for future work in this area.

**HERITABILITY STUDIES**

Heritability studies are used to assess the extent of the genetic contribution to a particular phenotype, i.e., the extent to which the disease phenotype is controlled by the host’s genetic make-up. In humans, this is most commonly done as twin studies, or more recently, with families and household contacts to determine the degree of concordance of genotype and phenotype (41). In animals, this can be more thoroughly investigated using breeding programs and experimental infection. Heritability is an important aspect of any disease and is an essential factor in the successful implementation of breeding programs. Imperfect diagnostics, such as the limited sensitivity of the skin test in BTB detection, raises the possibility that the true heritability of this disease may be even higher than observed (9). Historical data on differential BTB susceptibility in cattle breeds have been outlined by Vordermeier et al. (49) and will not be addressed here.

**Livestock**

Numerous studies have shown cattle breeds to display different levels of susceptibility to BTB. A study done by Ameni et al. (3) with Zebu, Zebu-Holstein crosses, and Holstein cattle under identical field husbandry conditions in Ethiopia showed both a higher prevalence and severity of BTB in the Holstein cattle compared with the Zebu or Zebu-Holstein crosses. Another study of Ethiopian cattle (8) identified breed as a significant factor affecting TB manifestation. Breed types were classified as local, exotic, or cross-bred and showed exotic cattle to have a higher risk of severe BTB pathology.

Bermingham et al. (7) investigated the heritability of single comparative intradermal tuberculin (SCIT) test responsiveness and confirmed M. bovis infection in Irish Holstein-Friesian dairy cattle. Approximately 600–800 herd tests encompassing 14,000 cows showed heritability estimates of 0.14 and 0.18 for SCIT test responsiveness and confirmed M. bovis infection, respectively. A similar study done on British Holstein-Friesian dairy cattle calculated heritability estimates of SCIT test responsiveness and confirmed M. bovis infection to be 0.16 and 0.18, respectively (12).

**Wildlife**

Red deer are found as both livestock and wildlife in various parts of New Zealand, Australia, the USA, and Canada. They are highly susceptible to BTB and act as a reservoir for transmission to cattle (21). A study by Mackintosh et al. (28) showed differential susceptibility and transmission in red deer, which was attributed to host genetic variation. Experimental infection of stags showed a range of disease phenotypes, which was closely mirrored in the response of sired offspring, suggesting a strong genetic basis for resistance. The heritability of BTB resistance was calculated to be as high as 0.48. An investigation of BTB in white-tailed deer in Michigan, USA, by Blanchong et al. (10), using microsatellites, found BTB-infected deer to be significantly more closely related than noninfected deer. The heritability studies performed in livestock and wildlife can be seen in Table 1.

**ASSOCIATION STUDIES**

Association studies compare the allele frequencies of specific markers between infected/diseased and uninfected/not
diseased individuals and are currently the most widely utilized in studying susceptibility to infection or disease. These case-control association studies are better than linkage studies at detecting genes that have a small effect (13), and as TB is known to be under polygenic control, there are likely to be many genes that each have a small effect on susceptibility. Genetic association studies can be roughly divided into two categories: candidate gene or whole genome studies. The candidate gene approach investigates single nucleotide polymorphisms (SNPs) or other variation in genes deemed to be of biological relevance to the question at hand. For example, in the case of TB, one might select genes involved in the immune response or that have been shown to be associated with other infectious diseases, even in other species. This approach utilizes a relatively small number of markers but can be expanded depending on budget and time. One of the main disadvantages of this approach, however, is that the marker associated with disease resistance may not be causative but may be in linkage disequilibrium (LD) with the causative variant. This is particularly applicable to those species with small effective population sizes and limited ranges, where LD blocks are likely to be longer (13). The whole genome approach involves genotyping polymorphic markers across the whole genome and, therefore, makes no prior assumptions as to which genes may or may not be involved. This can be a distinct advantage, as it is likely that there will be many more genes involved in disease resistance than those of the immune system. This approach thus utilizes a large number of markers and is more likely to identify novel genes or pathways involved in susceptibility (4). The candidate gene approach is less commonly employed in animals with assembled genomes, where sufficient data exist to interrogate the whole genome. However, in animals without sufficient genomic data, such as most wildlife species, candidate genes are a frequently utilized starting point, even though the implicit assumptions are that relatively few loci underpin the phenotype, and their effect is detectable.

Livestock

Kadarmideen et al. (24) investigated polymorphisms of the 3’-untranslated region microsatellite of the SLC11A1 (NRAMP1) gene and their association with BTB in African Zebu cattle. They compared four phenotypes, namely SCIT test responsiveness, presence of visible lung lesions, culture test outcome, and the predicted true infection using a Bayesian model, and found two alleles to be significantly associated with reduced incidence of BTB traits. Driscoll et al. (17) used microsatellite loci to analyze genetic factors in British cattle and found two markers, BMS2753 and INRA111, to be strongly associated with BTB reactor status. The BMS2753 marker lies in close proximity to IFNGR1 and several other immune response genes, and INRA111 was previously associated with mastitis (44). A genome-wide association scan of BTB susceptibility in Holstein-Friesian dairy cattle by Finlay et al. (19) found three SNPs in a region on BTA 22 to be associated with BTB susceptibility. This region contains the taurine transporter gene SLC6A6. Sun et al. (47) investigated the role of polymorphisms in the toll-like receptor (TLR) 1 and 9 genes with susceptibility to BTB in Chinese Holstein cattle. Significant associations were found with TLR1 genotype variants at TLR1-G1596A and BTB susceptibility and a trend toward significance with TLR1-A1475C.

Wildlife

The European wild boar is abundant on the Iberian peninsula, and its range and population size are increasing steadily. It has become a wildlife reservoir for BTB in regions of Spain, and concern has been raised about the spread and management of this disease in both wild and contained populations (1). Acevedo-Whitehouse et al. (1) investigated the genetic component of resistance in European wild boars from southern Spain and found that increased genetic heterozygosity had a significant positive effect on BTB resistance, in terms of both infection status and disease severity/dissemination. Several of the loci tested also revealed high homology to regions of the genome with immune-related functions. Naranjo et al. (34) investigated polymorphisms of a methylmalonyl-CoA mutase (MUT) microsatellite and their relationship to BTB susceptibility in European wild boar. The microsatellite is located next to exon 2 of the gene and exhibited three polymorphisms within the population. The authors found evidence of a balancing polymorphism, with one allele conferring significant protection, another conferring significant increased risk of infection, but the presence of both alleles conferring the highest level of protection against infection. African buffalo are a maintenance host of BTB in southern Africa and pose a risk of infection to cattle and surrounding communities and a threat to biodiversity and ecotourism (40). Le Roex et al. (40) explored polymorphisms in immune-related genes and their association with BTB susceptibility in the African buffalo with fluorescent genotyping. SNPs located in three genes, SLC7A13, IL10, and DMBT1, were found to be significantly associated with BTB status in the buffalo. The association studies performed in livestock and wildlife can be seen in Table 2.

Table 1. BTB heritability studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Animal</th>
<th>Breed</th>
<th>Factor</th>
<th>Result/Heritability Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mackintosh et al. (2000)</td>
<td>red deer</td>
<td>Zebu/Holstein/Zebu-Holstein</td>
<td>BTB resistance</td>
<td>0.48</td>
</tr>
<tr>
<td>Ameni et al. (2007)</td>
<td>cattle</td>
<td>Zebu/Holstein/Zebu-Holstein</td>
<td>BTB prevalence and severity</td>
<td>higher in Holstein</td>
</tr>
<tr>
<td>Blanchong et al. (2007)</td>
<td>white-tailed deer</td>
<td>Irish Holstein-Friesian</td>
<td>BTB infection</td>
<td>skin test response; M. bovis culture</td>
</tr>
<tr>
<td>Bingham et al. (2009)</td>
<td>cattle</td>
<td>British Holstein-Friesian</td>
<td>BTB infection</td>
<td>skin test response; M. bovis culture</td>
</tr>
<tr>
<td>Brotherton et al. (2010)</td>
<td>cattle</td>
<td>exotic/local/cross bred</td>
<td>BTB severity</td>
<td>higher in exotic breeds</td>
</tr>
</tbody>
</table>

BTB, bovine tuberculosis.
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Table 2. BTB association studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Animal</th>
<th>Breed</th>
<th>Associated Factor</th>
<th>Gene/Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acevedo-Whitehouse et al. (2005)</td>
<td>European wild boar</td>
<td></td>
<td>Infection status; BTB severity</td>
<td>heterozygosity associated with resistance</td>
</tr>
<tr>
<td>Naranjo et al. (2008)</td>
<td>European wild boar</td>
<td>multiple</td>
<td>lesions &amp; culture</td>
<td>methyalmalonyl CoA mutase</td>
</tr>
<tr>
<td>Driscoll et al. (2011)</td>
<td>cattle</td>
<td>African Zebu</td>
<td>skin test response</td>
<td>BMS2753; INRA111</td>
</tr>
<tr>
<td>Kadarmideen et al. (2011)</td>
<td>cattle</td>
<td></td>
<td>skin test response; visible lesions</td>
<td>SLC11A1</td>
</tr>
<tr>
<td>Finlay et al. (2012)</td>
<td>cattle</td>
<td>Irish Holstein-Friesian</td>
<td>EBV (based on skin test response)</td>
<td>Chr 22, SLC6A6</td>
</tr>
<tr>
<td>Sun et al. (2012)</td>
<td>cattle</td>
<td>Chinese Holstein</td>
<td>skin test response</td>
<td>TLR1</td>
</tr>
<tr>
<td>le Roex et al. (2013)</td>
<td>African buffalo</td>
<td></td>
<td>skin test/ELISA response</td>
<td>SLC7A13; IL1a; DMBT1</td>
</tr>
</tbody>
</table>

EBV, estimated breeding value.

actions need to be rigorously replicated and/or validated before being accepted as probable. To reduce the likelihood that spurious associations are identified, there are a number of considerations that must be taken into account.

Sample Collection and Phenotype Classification

The first consideration is the accuracy of the disease diagnosis. The best diagnostic tool available, i.e., the test with the highest specificity and sensitivity, should be used for designating individuals as cases or controls, as too many misclassifications could severely bias the association tests (2, 46). The phenotypes must also be clearly defined according to the question/objective at hand; for example, if one is interested only in active disease, the SCIT test alone may not be an appropriate diagnostic. The cases and controls should have comparable exposure to the pathogen in order to be correctly classified as having a susceptible or resistant phenotype. Cases and controls should also ideally be matched as closely as possible in all other ways (age, environment, etc.) so as not to introduce additional variables that may confound the association testing (46). The total number of samples in a study is an important consideration, often overlooked. This must be sufficiently large to generate enough statistical power to detect variants that have a relatively small effect, as is the usual situation in a complex disease. Statistical power is a function of the size of the study population, the allele frequency of the SNP, the size of the effect of the SNP, and the level of LD. Phenotype determination in wildlife may also be more difficult. Phenotype determination in wildlife may also be more difficult to ascertain. Many wildlife species have not had their full genomes sequenced, and thus the availability of genetic information is limited. This means that much work must be done prior to beginning a genetic association study. This includes the identification and development of suitable markers and, ideally, the elucidation of the complex genetic interactions and pathways that may operate within the species. Even in well-established models, such as cattle, many proteins and gene products have not been characterized, and much annotation is merely hypothesized rather than experimentally established. Associated markers may also not be the causal variant in a disease but, rather, may be in LD with the causal variant (13, 41). In the absence of established LD information for a particular species, this can be difficult to ascertain. Wildlife species are not managed in the same fashion that domestic livestock may be, and thus relatedness can be a concern. A lack of population ecology data on a particular species leaves researchers unable to establish the level of relatedness in a population, and this can introduce confounding effects. Phenotype determination in wildlife may also be more difficult due to absent/reduced diagnostic capabilities for many species and the logistical complications of capture and/or recapture in a natural environment.

Experimental Design and Analysis

The experimental design of a study is crucial in order to produce results that are not false positives or false negatives. Batch effects occur when different sets of samples are run on different days or in batches under slightly different conditions, caused by human error or different reagents or hardware and can result in spurious associations. If samples must be run in different batches, controlling for batch effects can be done by the randomization of samples and variables across batches. Population stratification is the other most common cause of spurious associations. If subgroups within the study population have different allele frequencies, associations may be found that are a function of the population substructure rather than disease susceptibility (13, 41). Any possible subgroups should be randomized across batches and controlled for during statistical analyses. In studies where large numbers of markers are compared across many genes, multiple comparisons are made. To minimize the occurrence of type I errors, correction for multiple testing, e.g., Bonferroni correction, should be applied where appropriate (41).

Wildlife

In studies involving wildlife species, additional factors may hamper a genetic study. Many wildlife species have not had their full genomes sequenced, and thus the availability of genetic information is limited. This means that much work must be done prior to beginning a genetic association study. This includes the identification and development of suitable markers and, ideally, the elucidation of the complex genetic interactions and pathways that may operate within the species. Even in well-established models, such as cattle, many proteins and gene products have not been characterized, and much annotation is merely hypothesized rather than experimentally established. Associated markers may also not be the causal variant in a disease but, rather, may be in LD with the causal variant (13, 41). In the absence of established LD information for a particular species, this can be difficult to ascertain. Wildlife species are not managed in the same fashion that domestic livestock may be, and thus relatedness can be a concern. A lack of population ecology data on a particular species leaves researchers unable to establish the level of relatedness in a population, and this can introduce confounding effects. Phenotype determination in wildlife may also be more difficult due to absent/reduced diagnostic capabilities for many species and the logistical complications of capture and/or recapture in a natural environment.

Additional Challenges

The type of association study should reflect the aim of the study, with candidate gene studies having greater power to detect small effects and genome-wide studies being better suited to identifying novel genes/pathways (4). In the same way that the host’s genetic make-up influences the outcome of infection, so too does the genetic make-up of the pathogen. Information regarding the genetic variation and lineage of the pathogen should be included, and reported, where known. BTB is influenced by many genes, and the role of gene-gene interactions and gene-environment interactions cannot be excluded.
A genetic variant not found to be associated on its own may still play a role in conjunction with another gene or variant, and the same may also be true for studies undertaken under different environmental conditions (41, 46). Current methodologies seldom include this type of analysis, and thus these genes are missed. The type of markers used in a study is also important: while microsatellite markers are often well characterized and work very well in linkage studies, the high allelic diversity and reduced LD at these loci may reduce the power to detect associations with a disease phenotype in a population-level study (16).

**GENE EXPRESSION STUDIES**

Studying the gene expression pattern of a particular phenotype provides another approach to identifying candidate genes. Gene expression studies look to identify gene transcripts that are differentially expressed in groups of animals to identify markers of infection. The primary goal is generally for use in diagnostics, but these studies can also inform genetic researchers of candidate genes worthy of investigation, as well as contributing information that can be used in conjunction with genetic and other data in a systems biology approach to build a more complete understanding of the pathways/systems involved in disease (14, 50). Gene expression studies will not be covered extensively in this review, as they are not strictly genetic studies, but nevertheless can be of great assistance. Studies using natural models of infection, i.e., that investigate differential gene expression in cells/tissues of animals that were naturally infected with BTB, are particularly useful, as this is the closest approximation of what might happen in nature.

Studies using natural BTB infection in cattle have identified many genes that are differentially expressed between *M. bovis*-infected and uninfected animals; these include, but are not limited to: ADAM17, CXCR3, IER5, PHB2, CD84, TBK1, TLR2, TLR3, BCL2, Nfatc4, IFNG, Ifng1, Tnfsf13b, Kliaa1971, Slamf1, Casp1, Deftb10, Ifnar1, Kirs3ds1, Myd88, Ptprn2, Stat1, Stat2, Trem1, Tyk2, Tyrobp, Cd83, CtlA, Il1a, Il8, and Il15 (11, 25, 29, 30). In wildlife, studies of naturally infected wild boar have identified the differential expression of many genes, including C3, Mtu, Anx, Cul, Vcam-1, C7, Arg, Opn, Cxcr4, Vdr, Ba29, Gal-1, Sla class I and II, Ig, Cc1qB (20, 35, 36). A gene expression study in Iberian red deer showed the differential expression of SMAP-29 and CK2 between *M. bovis*-infected and uninfected individuals (18). It is evident that a number of the genes listed above are involved in the immune system, many of which, such as VDR, IFNG, and TLR2, are also implicated in human TB association studies (6, 33, 42), lending further credence to the identification of the gene and its product as an important factor in mycobacterial disease.

**CONCLUSIONS AND FUTURE DIRECTIONS**

The identification of genetic markers linked to a resistant BTB phenotype can inform studies in other species and may enable the breeding of animals with greater disease resistance. This would, at the very least, be a useful additional measure in conjunction with the other control strategies currently available. The ability to breed animals with a greater BTB resistance may be of considerable commercial benefit to the cattle farming industry and would certainly be invaluable in other species, such as the African buffalo, which forms part of a multimillion dollar hunting and breeding industry in South Africa, with “disease-free” buffalo particularly prized. This also pertains to other wildlife species kept in a farm or reserve environment, where selective breeding can produce very tangible benefits. Allen et al. (2) suggest that several other bovine pathogens such as *Brucella abortus* and *Mycobacterium avium paratuberculosis* may operate within their hosts in a similar manner to *M. bovis*, and thus the breeding of BTB resistance may also inadvertently select for resistance to those pathogens as well. However, it is also possible that such animals may show increased susceptibility to other diseases.

To date, most of the genetic research has focused on heritability and candidate gene association studies investigating single markers. While association studies remain the most popular choice in disease susceptibility studies, there are a number of considerations to be taken into account in the collection and classification of samples, the experimental design and analyses, as well as the species under investigation. Current human studies in TB susceptibility have included a number of genome-wide association studies, and this may be a direction future animal work will take, provided an assembled genome of the animal of interest is available. The large number of subjects required and the relatively low power of genome-wide association studies to detect associations of small effect do hamper this approach, but the identification of novel loci may become increasingly important (23). Gene expression studies, particularly of natural infections, provide an excellent pool of differentially expressed candidate genes for selection and should be utilized to inform genetic studies wherever possible.

Future genetic work may also need to progress from the strategy of looking for individual loci. There are three main approaches to investigating genetic markers and their associations: 1) to investigate individual markers in isolation and their association with the biological phenotype, 2) to investigate gene-gene interactions by testing marker/SNP combinations for association with the phenotype, and 3) to investigate pathways and determine whether a group of related genes/markers are associated with the phenotype. As we know, genes do not work in isolation, and there is increasing interest in the analysis of gene-gene interactions and pathways. Investigators have begun to study these processes in humans (48, 51), but this has yet to be embarked upon in animal disease studies.

In summary, investigations into the role of the host’s genetic make-up in BTB susceptibility are in their infancy, and there is an enormous amount of work still to be done before any true understanding of the interplay between host genetics and BTB susceptibility can be definitively achieved. The genome assembly of a growing number of species will provide researchers with the tools and information needed to tackle this imposing question from a number of angles, and the cooperation of multiple disciplines will ensure the best success at identifying the host genetic component of BTB resistance.

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