chromatin but also an independent process involving the recruitment of histone deacetylases (HDACs). Furthermore, this latter process appears to be the dominant gene-silencing pathway used for the region of the genome involved in the mating type switch (Yamada et al., 2005). Also, dissection of the RNAi mechanism of gene silencing in *S. pombe* shows that the exosome and in particular an associated polyA polymerase (Cid14) are required for gene silencing (Buhler et al., 2007). The fact that silencing of the *PHO84* gene involves both the exosome (Rrp6) and HDACs strikingly parallels these aspects of silencing in *S. pombe*. Indeed, in using both RNAi and HDAC-associated pathways for gene silencing, *S. pombe* demonstrates its evolutionary position at center stage between *S. cerevisiae* and mammals.

As with all advances, new answers raise new questions. First, it is important to know how many other genes in *S. cerevisiae* besides *PHO84* are subject to a similar process of stress-induced gene silencing. Clearly, expression array analysis is called for here. Also, from an evolutionary perspective it is interesting that for *S. pombe* and also possibly in plants, RNAi-induced gene silencing is the predominant use of RNAi. In contrast, in higher eukaryotes, RNAi appears to primarily downregulate mRNA expression and inhibit translation efficiency in the cytoplasm via the actions of siRNAs and microRNAs. Apparently, there are no clear protein homologs in *S. cerevisiae* of the well-defined RNAi apparatus in *S. pombe* and higher eukaryotes. However, some surprises may await us that may reveal a more unifying mechanism for all eukaryotic gene silencing. Clearly, the rapid degradation of newly synthesized CUTs, both sense and antisense, is a key aspect of gene silencing in eukaryotes.

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RecBCD: The Supercar of DNA Repair

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DOI 10.1016/j.cell.2007.11.004

The DNA helicase RecBCD pauses when it reaches recombination hotspots known as Chi sites and then proceeds at a slower speed of translocation than before Chi recognition. Reporting in this issue, Spies et al. (2007) now show that this reduction in translocation velocity occurs when RecBCD changes which of its two motor subunits is in the lead.

Motor car fans will be familiar with the Bugatti Veyron, until a few weeks ago the world's fastest supercar. The Veyron is an astonishing engineering achievement powered by the fusion of two V8 engines to create a single W16 quad-turbocharged motor that produces 1001 bhp and is capable of speeds of over 250 mph. Many readers of *Cell*, however, may be more familiar with the "supercar" of DNA repair, RecBCD. This enzyme is responsible for initiating repair of double-strand breaks in many bacteria. Like the Veyron, RecBCD contains two engines (the RecB and RecD helicase motor subunits; see Figure 1) that are capable of driving the complex along DNA at over 1000 base pairs per second. The RecB and RecD motors are each powered by hydrolysis of ATP, the combination consuming two ATP molecules per base pair. Significantly, RecBCD is more cleverly engineered than the Veyron because the two motors can work independently. In fact, in work presented in this issue, Spies et al. (2007) show that following the recognition of recombination hotspots called Chi (crossover *h*otspot *i*nstigator) sites, RecBCD is able to switch which of its two motors takes the lead and thereby regulate the translocation velocity of the complex.

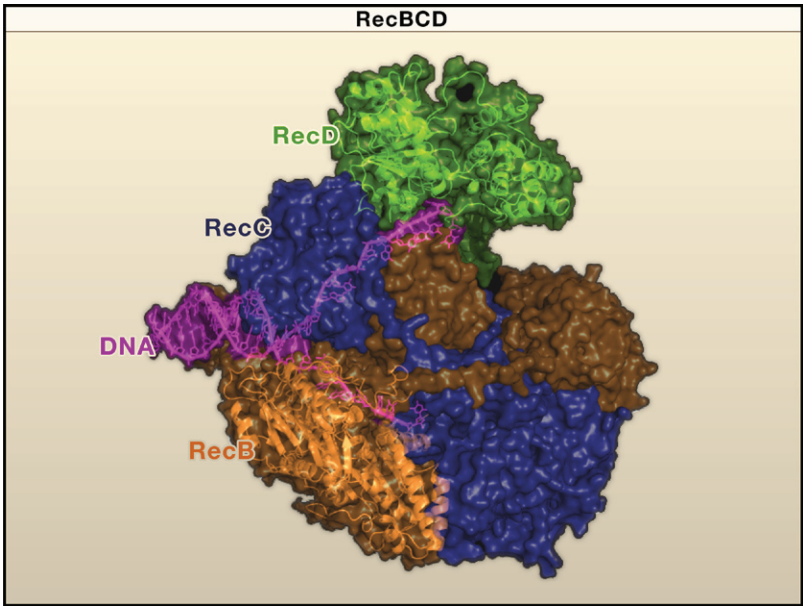

Figure 1. The Twin Motors of RecBCD
Shown is the crystal structure of RecBCD in a complex with a long DNA substrate that contacts each of the two motor subunits (RecB and RecD). A transparent molecular surface is shown with the RecB and RecD motor domains overlaid in ribbon representation (K. Saikrishnan and D.B.W., unpublished data).

In addition to a molecular motor, the RecB subunit also contains a nuclease domain that digests the DNA duplex as it goes along. This mode of operation seems to be used by bacteria as a defense against invading phage DNA, which is cut by restriction enzymes and then digested by RecBCD. For repair of double-strand breaks, however, a different mode is initiated due to the presence of Chi sites, eight base pair sequences that are overrepresented in the *Escherichia coli* genome. When RecBCD encounters Chi, the nuclease activity of the enzyme is regulated to produce a 3′-tailed duplex onto which the RecA protein is loaded to initiate repair by homologous recombination. The apparent simplicity of the overall reaction belies the underlying complexity of the events that take place during the catalytic cycle.

The crystal structure of RecBCD (Singleton et al., 2004) revealed how the three proteins were assembled and showed that each of the motor subunits contacted a single strand of the DNA substrate (Figure 1). Although the crystal structure provided fascinating insights into a number of aspects of RecBCD function, the structure is a single snapshot in a very complex pathway and many aspects of the mechanism remain unclear. In particular, the recognition and response of RecBCD on encountering Chi are not addressed by the structure. Consequently, Kowalczykowski, Spies, and their colleagues have been using single-molecule techniques to gain insight into this aspect of the mechanism. Their initial studies (Spies et al., 2003) revealed that Chi acts as a "molecular throttle" that regulates the speed of DNA translocation by the RecBCD motors. Upon encountering Chi, the enzyme pauses for several seconds and then continues to translocate along the DNA but at approximately half the original speed, leading the authors to suggest that the faster (RecD) motor had become uncoupled in some way as a consequence of interaction with Chi. Similar ideas that went even further were proposed a number of years ago by Stahl and coworkers (Myers et al., 1995) based on genetic data, which suggested that after Chi recognition, RecBCD converts to a state resembling a complex that lacks RecD entirely (i.e., RecBC). This led to the idea that the RecD subunit might dissociate from the complex after Chi (known as the RecD "ejection" hypothesis), an idea that remained contentious for a number of years. However, single-molecule studies again provided the answer and showed that RecD in fact remains a part of the enzyme complex post-Chi (Handa et al., 2005). So what actually happens to RecBCD post-Chi and, in particular, what happens to RecD? Spies et al. (2007) now provide the answer.

When initially translocating along DNA, the two motors of RecBCD are not running at equal speeds. The RecD motor runs faster than RecB and so leads the complex. As RecB struggles to keep up, a loop of single-stranded DNA (ssDNA) from the 3′ strand spools out ahead of the complex. Upon encountering Chi, the enzyme pauses for a few seconds before continuing at approximately half the pre-Chi rate. During this pause, at least two things take place. First, the spooled ssDNA is reeled in by the RecB subunit. Once the loop has been pulled in, the complex continues to unwind the DNA duplex but now with the slower RecB subunit as the leading motor with a consequent reduction in translocation speed. The second event is that some sort of conformational change (presumably) takes place, the result of which is uncoupling of the RecD motor. Importantly, Spies et al. show that a mutant enzyme complex in which the RecD motor is inactivated still pauses at Chi before proceeding at the same initial rate as observed prior to Chi, and that rate on average is similar to the post-Chi rate for the wild-type complex. This result shows that the pause cannot simply be due to reeling in of the ssDNA loop because no such loop would be formed with this mutant RecBCD complex.

One puzzling aspect of this mechanism is that the enzyme seems to be overengineered. Why bother to go to all of this trouble just to reduce the translocation speed by a factor of two? Spies et al. suggest that a more slowly translocating complex may be better suited for the subsequent process of RecA loading and the initiation of recombination. Although this may be an explanation, their single-molecule experiments reveal that the intrinsic variation in pre-Chi translo-

cation rates for individual RecBCD complexes is as much as 8-fold, considerably greater than the 2-fold reduction induced by Chi. One is left with the suspicion that we must be missing something more fundamental here, most likely some aspect of RecBCD regulation.

Several other questions remain unanswered. Does RecD disengage from the substrate or simply slow down to match the speed of RecB and, in either case, what is the physical manifestation of that process and how does that relate to the pause at Chi? After Chi, does RecD continue to hydrolyze ATP or even to bind to the translocating DNA? If not, then these functions must be physically prevented in some way. Finally, what changes in the enzyme initiate loading of RecA protein onto the DNA? Further work, possibly including further crystal structures, will be needed to answer these questions.

It is perhaps sobering to end with the observation that if RecBCD were scaled up to the size of a supercar it would travel at a speed of over 500 mph, knocking the Veyron's measly 254 mph into a cocked hat. It seems that mankind still has much to learn from nature.

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