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DNA loops specify p53 network responses

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Genome-wide chromatin interaction studies demonstrate that chromosomes are organized in distinct domains, or territories, which are somehow re-established after cell division. The mechanisms that specify these ordered chromatin topologies remain fairly elusive, but functional studies have established that long-range chromosomal interactions within territories, but also between territories, contribute to gene expression in development and disease. Recently, we employed *Drosophila* genetics to present in vivo evidence that long-range interactions between a p53 enhancer and p53 target genes are required for proper transcriptional regulation.[1] Using tailor-made deletions, we found that a single p53 enhancer specifies stimulus-dependent regulation of multiple targets in cis within a 330 kb region known as the Reaper region. More surprising, however, was that these effects were also observed at greater distances. Specifically, this same p53 element was required for proper regulation of genetically unlinked targets residing 20 Mb across a centromere and additional targets on other chromosomes.

Removal of the enhancer in our study did not abolish all interactions with distant target genes. In animals lacking the enhancer region, sequences directly outside the deletion retained the ability to contact targets within the Reaper region, but interaction frequencies became more variable. Therefore, in addition to sequences within the p53 enhancer region, other DNA elements probably help organize the Reaper region territory. However, from a non-native chromosome, the fly p53 enhancer was able to partially contact and restore regulation to some target genes residing in trans. The ability to restore trans looping contacts from a foreign site demonstrated that determinants of trans chromatin contacts might be modular and perhaps be mediated through sequence motifs, secondary structures, or epigenetic features. We do not yet know the minimally obligate sequences necessary to produce a contact in trans. Thus, future studies using additional rescue fragments may elucidate features required for proper p53 target gene regulation. Mutations encompassing key protein binding sites such as transcription factors, insulators, or other DNA binding proteins may identify the mechanisms that mediate long-range interactions between the p53 enhancer region and its targets.

We found that contacts were generally unaffected by p53 status or DNA damage, a stimulus that activates p53 and its target genes. Because links were detected without the need for a genotoxic stimulus, we favor the idea that specific and distinct looping patterns could reflect pre-programmed conformations poised to control alternate p53 targets, thereby enabling distinct cellular responses. For example, differentially pre-configured assemblies in different cell types might specify tissue-specific, p53-dependent behaviors such as apoptosis or cell cycle arrest. A useful analogy here is the client–server model in computer networks, since many target genes (clients) could potentially be controlled by a single enhancer (server). Likewise, certain looping contacts might be shared among all cells, while others might be tissue-specific. Both models are consistent with our data, but, ultimately, comprehensive information at single-cell resolution will be needed to fully evaluate these scenarios. High-resolution, quantitative FISH on specific cell types will help resolve this issue in the future.

Interestingly, p53 enhancer mutants manifested a diverse array of congenital defects not seen in control animals, and the scope of congenital phenotypes was not shared with *p53*$^{-}$ animals. Hence, elimination of the p53 enhancer caused disruptions that go beyond proximal regulatory effects and probably reflect interference with other regulatory programs. Future experiments mapping all contacts to the p53 enhancer in vivo using genome-wide chromosome interaction techniques may clarify these programs. These phenotypes also raise the possibility that, in some cases, developmental networks can more effectively compensate for the absence of a trans factor than the absence of an enhancer element and surrounding sequences.

We established that a canonically defined *Drosophila* p53 enhancer regulates intra- and inter-chromosomal targets through direct long-range associations in *cis* and in *trans*. Future studies will assess whether this property is shared by all p53 enhancers. Likewise, other enhancers, together with their cognate binding factors, could also exert analogous regulatory activity in trans. If broadly generalized, this precedent could offer a framework that helps explain genetic diseases caused by lesions in non-coding sequences and the appearance of stereotyped translocations in human cancers.

Reference

1. Link N, et al. Genes Dev 2013; 27:2433-8; PMID:24240233; http://dx.doi.org/10.1101/gad.225565.113

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