

Nucleolar Dominance

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Nucleolar dominance is an epigenetic phenomenon in plant and animal hybrids that describes the failure to form nucleoli, the sites of ribosome synthesis, on chromosomes inherited from one parent. The molecular basis for nucleolar dominance is the reversible silencing of ribosomal RNA genes transcribed by RNA polymerase I. These genes are clustered at loci spanning millions of base pairs, making nucleolar dominance one of the most extensive known chromosomal-silencing phenomena.

Advanced

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Introduction

0976.1 Nucleolar dominance is a common phenomenon in hybrid plants and has also been studied in hybrid frogs (*Xenopus*), hybrid flies (*Drosophila*), and mammalian somatic cell hybrids. Nucleolar dominance was among the first epigenetic phenomena to be described and involves reversible gene-silencing on a scale perhaps second only to the inactivation of one X chromosome in somatic cells of female mammals. Like X-inactivation, which is thought to be a mechanism for equalizing X-linked gene expression in females and males, nucleolar dominance is likely to be a manifestation of a dosage compensation mechanism that controls the fraction of ribosomal ribonucleic acid (rRNA) genes that are active under a given set of developmental and physiological conditions. (See A0795.)

0976.2 rRNA transcription is accomplished by RNA polymerase I (RNA pol I). In almost all eucaryotes, RNA pol I has only one function: the synthesis of transcripts that are processed to form the 18S, 5.8S and 25–28S (the size is species-dependent) RNAs of cytoplasmic ribosomes. Hypotheses to explain nucleolar dominance include preferential activation of the dominant rRNA genes, based on RNA pol I transcription factor availability and/or selective repression of the underdominant (inactive) set of rRNA genes, based on unknown genic or chromosomal cues that allow parental sets of rRNA genes to be discriminated.

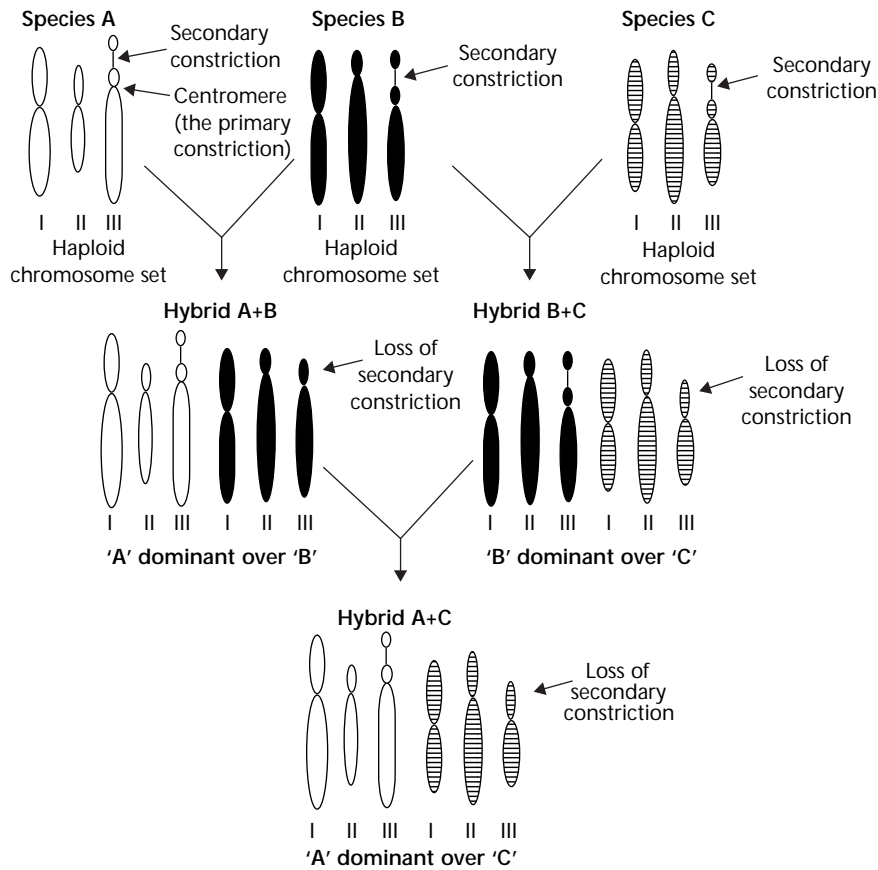
Brief History

0976.3 Nucleolar dominance was first described as a change in chromosome morphology. Navashin noted that in numerous species of the plant genus *Crepis* there was always one chromosome pair that displayed secondary constrictions at metaphase. When crossed, 8 of 21

species combinations yielded F₁ hybrids with secondary constrictions on chromosomes inherited from both parents (Navashin, 1934). In the other 13 hybrids, secondary constrictions formed on the chromosomes inherited from only one parent (**Figure 1**), a phenomenon Navashin named ‘differential amphiplasty’. Secondary constrictions were always absent from chromosomes of the same species regardless of whether that species served as the maternal or paternal parent. However, in F₂ segregants that essentially recreated the underdominant species (the species whose secondary constrictions were suppressed), secondary constrictions were again formed on both diploid copies of the chromosome. This showed that the affected loci had not been lost or permanently altered in the hybrid, but that reversible interactions among the parental genomes were somehow responsible for the unusual chromosome behavior.

Navashin’s contemporary, McClintock demon- 0976.4 strated that the secondary constriction in maize corresponds to the chromosomal locus where the nucleolus is formed (McClintock, 1934). She named this locus the ‘nucleolar organizer’, a term which is still in use today, though in slightly modified form (nucleolus organizer region, NOR). McClintock offered an interpretation of Navashin’s data, noting that the *Crepis* species tested could be arranged in a hierarchy of NOR dominance, species in top tiers being dominant over all species below. McClintock proposed that NORs compete for something present in the cell in limiting quantities and that some NORs are better competitors than others.

In the 1960s, NORs were shown to be the 0976.5 chromosomal loci where the rRNA genes are clustered by the hundreds (sometimes thousands), suggesting that differential amphiplasty might result from expressing only one parental set of rRNA genes (Wallace and Langridge, 1971). Indeed, using newly developed molecular hybridization techniques, Honjo and



0976.F001 **Figure 1** Discovery of nucleolar dominance as the hybridization-induced absence of a secondary constriction at the nucleolus organizer region (NOR) of metaphase chromosomes. Based on the observations of Navashin and McClintock, haploid chromosome sets of three 'pure' species and their hybrids are shown. Each species has a chromosome with a NOR that forms a secondary constriction at metaphase. In hybrids, often the NOR from only one progenitor forms the characteristic secondary constriction. In the example shown, the NOR of species A is dominant in an A–B hybrid, the NOR of species B is dominant in a B–C hybrid and the NOR of species A is dominant in an A–C hybrid. Only actively transcribed NORs form a secondary constriction at metaphase, apparently due to a physical or enzymatic function of the nucleolus interfering with chromosome condensation.

Reeder showed that during the early development of *Xenopus laevis* × *X. borealis* hybrids, only *X. laevis* ribosomal RNAs could be detected (Honjo and Reeder, 1973). These authors appear to have introduced the term 'nucleolar dominance' to the literature. Interestingly, the *X. borealis* rRNA genes inactivated during early development in hybrids were expressed in adult organs and tissues. Likewise, in hybrids of the plant genus *Brassica*, the parental set of rRNA genes that is inactive during vegetative development is transcribed upon the transition to reproductive development (Chen and Pikaard, 1997b). In both *Xenopus* and *Brassica*, the inactive genes are free of associated RNA pol I, showing that regulation is controlled at the level of RNA synthesis rather than RNA degradation (Chen and Pikaard, 1997a; Honjo and Reeder, 1973). (See A0008.)

Possible Mechanisms

Ribosomal RNA genes evolve rapidly, as do the RNA pol I transcription factors that recognize them. For instance, a human cell extract will not transcribe a mouse rRNA gene promoter, nor will a mouse cell extract transcribe a human rRNA gene promoter. Divergence of a single transcription factor can account for the species-specificity of rRNA gene transcription in this case. If the human version of this factor is added to a mouse extract, the human rRNA gene promoter can program transcription. Likewise if the mouse version of the factor is added to a human cell extract, the mouse rRNA gene promoter will be recognized. Based on these results, silencing of a gene encoding a species-specific transcription factor in a hybrid could conceivably inactivate one set of ribosomal RNA

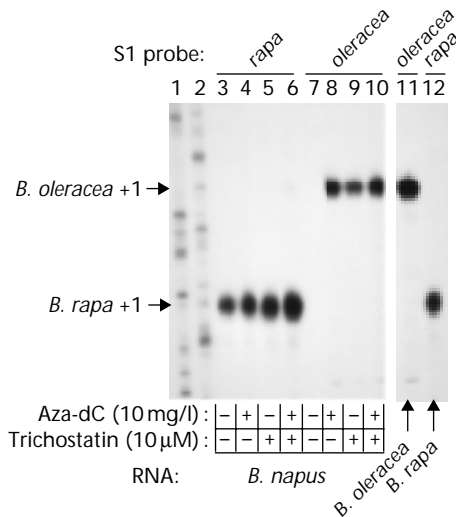


Figure 2 A silent set of ribosomal (rRNA) genes subjected to nucleolar dominance can be derepressed by 5-aza-2'-deoxycytosine (Aza-dC), an inhibitor of cytosine methylation or by trichostatin A (trichostatin), an inhibitor of histone deacetylation. This is demonstrated using *Brassica napus*, the allotetraploid hybrid of *B. rapa* and *B. oleracea*, in which rRNA genes inherited from *B. oleracea* are repressed (compare lanes 3 and 7). *B. napus* seeds were germinated on a medium containing no additions (lanes 3 and 7), or on a medium containing 5-aza-2'-deoxycytosine (lanes 4 and 8), trichostatin A (lanes 5 and 9), or both chemicals (lanes 6 and 10). Plants were harvested after 2 weeks in culture and an equal aliquot of RNA from each treatment was hybridized to *B. rapa* (lanes 3–6, 12) or *B. oleracea*-specific (lanes 7–11) probes and subjected to S1 nuclease protection analysis to detect transcripts from the genes inherited from the two progenitors. RNA isolated from *B. oleracea* and *B. rapa* (lanes 11 and 12) served as controls. Lanes 1 and 2 show sequencing ladders used as size markers. Note that 5-aza-2'-deoxycytosine and trichostatin A together are not significantly more effective at derepressing the *B. oleracea* genes than is either chemical alone, suggesting that DNA methylation and histone deacetylation are partners in the same repression pathway. (Reprinted with permission from Chen and Pikaard, 1997a.)

genes. This hypothesis may explain the observation that, in cell lines created by fusing mouse and human somatic cells, it is common for the human rRNA genes or the mouse rRNA genes to be transcribed, but not both. Presumably inactivation or loss of the gene(s) encoding the mouse or human species-specific transcription factor is responsible for this phenomenon (Reeder, 1985). However, in species closely related enough to interbreed, such as species within the plant genera *Brassica* or *Arabidopsis*, this hypothesis cannot explain nucleolar dominance because the pol I transcription machineries of the two parental species are compatible (Frieman *et al.*, 1999). Thus species-specific transcription factor availability is unlikely to explain nucleolar dominance in natural hybrids.

Experiments in *Xenopus* suggest that nucleolar dominance could result from competition for a transcription factor that can be used by both parental sets of rRNA genes. The dominance of *X. laevis* rRNA genes over *X. borealis* rRNA genes observed in hybrids can be mimicked using minigenes injected into frog oocytes (Reeder and Roan, 1984). When an *X. laevis* minigene is coinjected with an *X. borealis* minigene, the *X. laevis* rRNA gene is preferentially transcribed. Dominance in the oocyte injection assay is not due to differences in the gene promoters but to differences in the intergenic spacers located upstream of the gene promoters. These intergenic spacers in *Xenopus*, and all higher eucaryotes, contain repeated DNA sequences that, in *Xenopus*, enhance transcription from the adjacent promoter. Differences in enhancer number in *X. laevis* and *X. borealis* rRNA genes results in the preferential transcription of *X. laevis* rRNA genes, presumably due to preferential recruitment of one or more transcription factors. Based on indirect evidence, this 'enhancer imbalance' hypothesis was also proposed as a possible explanation for nucleolar dominance in plants such as wheat. However, tests of this hypothesis in *Brassica* and *Arabidopsis* have failed to reveal differences in the ability of dominant and underdominant rRNA genes to recruit transcription factors, as predicted by the hypothesis (Frieman *et al.*, 1999), pointing instead to chromosomal controls.

Consistent with the idea that nucleolar dominance is controlled at a chromosomal level, selective rRNA gene repression in plants has been shown to involve DNA (cytosine) methylation and histone deacetylation, chromatin modifications that can also silence protein-coding genes (Chen and Pikaard, 1997a). However, the mechanisms by which one set of rRNA genes are selected for repression are not known. Also unknown are the identities of key methylated DNA sequences, the DNA methyltransferases that carry out these methylation events, and the histone deacetylases that mediate rRNA gene repression.

Importantly, gene silencing in nucleolar dominance has been shown to be restricted to NORs and to not affect adjacent genes, indicating that nucleolar dominance is not a consequence of a larger chromosome silencing phenomenon (Lewis and Pikaard, 2001). However, NORs are not fully autonomous, such that chromosome rearrangements that move NORs to new chromosomal locations, or that delete adjacent sequences, can disrupt NOR silencing (Durica and Krider, 1978; Viera *et al.*, 1990). The fact that the chromosomal context of an NOR affects nucleolar dominance suggests that rRNA gene sequence differences are not sufficient to dictate which genes are dominant or underdominant. Apparently, other chromosomal cues are involved in the discrimination mechanism.

Summary

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Nucleolar dominance is a large-scale gene-silencing phenomenon restricted to the nucleolus organizer regions where rRNA genes are transcribed by RNA pol I. The unit of regulation in nucleolar dominance is not clear, but could be each individual rRNA gene or the NOR as a whole. The mechanism by which the two parental sets of rRNA genes are discriminated within the nucleus is also unclear and might involve positional cues established by the chromosomes on which the NORs are located. As is the case for other epigenetic phenomena, including X-inactivation and gametic imprinting, nucleolar dominance involves reversible, chromatin-mediated alterations in gene expression. However, unlike X-inactivation in somatic cells, chromosome choice is not random and, unlike gametic imprinting, maternal or paternal effects do not dictate the set of rRNA genes to be silenced. For these reasons, understanding nucleolar dominance may ultimately reveal novel mechanisms by which alleles and chromosomes are discriminated. (See A0279; A0686; A0768.)

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Further Reading

Glossary

Epigenetic phenomena: Heritable (or propagated) alternative states of gene expression, molecular function or organization specified by the same genetic instructions.

Gene promoter: The region of a gene that specifies where ribonucleic acid (RNA) polymerases will bind and RNA synthesis will begin.

Nuclear run-on: A technique in which RNA polymerases associated with deoxyribonucleic acid (DNA) at the time cells are broken open

and nuclei isolated are permitted to continue transcription using radioactive RNA precursors. The technique is used to determine whether the absence of an RNA is due to the absence of transcription.

Transcription: The synthesis of RNA from a DNA template; accomplished by enzymes known as RNA polymerases.

Transcription factor: A protein or group of tightly associated proteins that help RNA polymerases carry out transcription.

Keywords

gene silencing, epigenetic phenomena, ribosomal RNA, RNA polymerase I, nucleolus organizer

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