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This Week's Citation Classic

Brown D D & Dawid I B. Specific gene amplification in oocytes.
Science **160**:272-80, 1968.
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The genes for 18S and 28S ribosomal RNA are amplified specifically in oocyte nuclei of amphibians forming more than a thousand nucleoli in each nucleus. These extra genes support enormous rates of ribosomal RNA synthesis during oogenesis. [The *SCI*® indicates that this paper has been cited in over 530 publications since 1968.]

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"This paper and one published independently at the same time by Joseph Gall[1] were the first to demonstrate specific gene amplification—an event programmed into the development of a cell. The genes are those for ribosomal RNA in oocytes of the amphibian *Xenopus laevis*.

"My own involvement in this kind of research dates from 1960 when I first began to study gene expression in frog embryos at the Carnegie Institution's department of embryology in Baltimore. It had just become possible to measure RNAs as direct gene products, and the first RNAs to be purified were the stable ribosomal and transfer RNAs that comprise the bulk of cellular RNA. In 1964, John Gurdon and I found that a mutant of *Xenopus* that affects the number of nucleoli was defective in the synthesis of the two large ribosomal RNAs.[2] This confirmed that the nucleolus is the site of synthesis of ribosomal RNAs, a correlation that had already been made cytologically. Max Birnstiel, then in Edinburgh, demonstrated that this mutation was a deletion of the several hundred genes that encode these two ribosomal RNAs.[3] This crucial paper set the stage for the isolation of these genes, the very first instance of gene isolation from any living organism.

"An international meeting on the nucleolus was held in Montevideo, Uruguay, in 1965. Without a doubt, the highlight of that meeting was Birnstiel's demonstration of how he had used physicochemical techniques to isolate the ribosomal RNA genes.[4] At that conference I heard Oscar Miller, then a staff member at the Oak Ridge Laboratories, describe the presence of circular chromosomes in the many nucleoli of frog oocyte nuclei.[5] I knew instantly from the previous correlations of ribosomal RNA genes and the nucleolus that these must be extra copies of ribosomal RNA genes. Igor Dawid, a fellow staff member at Carnegie, and I set out to prove this idea.

"A key experiment described in our *Science* paper depended upon the isolation by hand of ten thousand nuclei from *Xenopus laevis* oocytes, a technique perfected by Mrs. Eddie Jordan, my colleague at Carnegie. We used buoyant density and hybridization methods to identify the amplified genes in extracts from these nuclei. Oocytes of amphibians, fish, and certain insects are known to amplify their ribosomal RNA genes, and there are now examples of amplification of genes for proteins. A phenomenon that I termed 'forced gene amplification' is a response by which cells become resistant to a drug by amplifying the gene whose product is interfered with by the drug. It is apparent that this is an important cause for resistance to chemotherapy. Our own search for other genes that might be amplified during development was negative causing us to focus on other kinds of gene control during development.

"The reference has been cited because it represents the first observation of one important mechanism for gene regulation in animal cells. Since that time, there have been numerous other examples of gene amplification in other animal cells. (For a review, see reference 6.)"

1. **Gall J G.** Differential synthesis of the genes for ribosomal RNA during amphibian oögenesis.
Proc. Nat. Acad. Sci. US **60**:553-60, 1968.
[The *SCI* indicates that this paper has been cited in over 280 publications since 1968.]
2. **Brown D D & Gurdon J B.** Absence of ribosomal RNA synthesis in the anucleolate mutant of *Xenopus laevis*.
Proc. Nat. Acad. Sci. US **51**:139-46, 1964.
3. **Wallace H & Birnstiel M L.** Ribosomal cistrons and the nucleolar organizer.
Biochim. Biophys. Acta **114**:296-310, 1966.
4. **Birnstiel M L, Wallace H, Sbrin J L & Fischberg M.** Localization of the ribosomal DNA complements in the nucleolar organizer region of *Xenopus laevis*. *Nat. Cancer Inst. Monogr.* **23**:431-47, 1966.
5. **Miller O L, Jr.** Structure and composition of peripheral nucleoli of salamander oocytes.
Nat. Cancer Inst. Monogr. **23**:53-66, 1966.
6. **Brown D D.** Gene expression in eukaryotes. *Science* **211**:667-74, 1981.