Profile of Michael Karin

Chronic inflammation can cause cells to ignore the call of apoptosis and instead proliferate unchecked, starting down the path to cancer. The transcription factor NF-κB, an immune system modulator, can act as one of the faulty links in this chain, with its continued presence not only preventing cell death but also encouraging the production of cellular growth factors. Michael Karin, Professor of Pharmacology at the University of California, San Diego (UCSD; La Jolla, CA) and elected to the National Academy of Sciences (NAS) in 2005, has been investigating the link between inflammation and cancer by studying NF-κB and its regulation in cells gone awry. In his Inaugural Article in a recent issue of PNAS (1), Karin further explored the surprising role of NF-κB in a mouse model of liver cancer. The absence of NF-κB was shown to increase the rate of hepatocarcinogenesis, an effect modulated by sustained activation of Jun kinase 1 (JNK1), a protein kinase whose activity is indirectly modulated by NF-κB.

Michael Karin

From Tel Aviv to L.A.

Born in Tel Aviv, Israel, in 1951, Karin first became interested in science in high school, where biology and chemistry both captured his attention. His high school, Municipal High School D (Tel Aviv, Israel), produced another NAS member, Adi Shamir, who was also elected in 2005. After high school, Karin wanted to pursue science and earn a Ph.D., and he entered Tel Aviv University (Tel Aviv, Israel) in 1972, where he continued to indulge his scientific interests with a double major in biology and microbiology. At the university, he worked on several projects, including one on bacterial genetics, where Karin developed methods of enriching for auxotrophic mutants in *Escherichia coli*. He remembers that professor Ezra Yagil strongly supported his interest in molecular genetics, encouraging Karin to continue his research studies. "He strongly encouraged me to study abroad," says Karin, who graduated magna cum laude in 1975.

At the time, options for pursuing molecular biology in Israel were not on par with those in the United States, so Karin decided to go to the United States for his Ph.D. He made the decision, in part, because knew that he could earn a Ph.D. in 4 years, much faster than is usually the case in Israel. Karin applied to universities on the east and west coasts and settled on the University of California, Los Angeles (UCLA). "I'd never been to the U.S.A.," he says, "but I knew about California and Los Angeles from the movies." In 1975, Karin entered UCLA to begin graduate school, and although interested in molecular biology, he did not have his eye set on a specific research path.

"In the beginning, I was not all that sure. I thought that I was interested in brain research," he says. Karin completed several research rotations with an emphasis on neurobiology, but he remembers one that did not go so well. "One professor was not so encouraging after I broke his pH electrode—three times," says Karin. That same professor did not have the money to support a student, so Karin moved on to the laboratory of Harvey Herschman. Even though a portion of Herschman's research focused on neurobiology, the project available to Karin was not in that area. "I was not so happy with it at the time," Karin says, "but it turned out to be my lucky break." Herschman assigned Karin to study the genetic regulation of metallothioneins. "That kind of seeded my future studying genetic regulation," says Karin.

Metallothioneins are low-molecular-weight proteins that protect cells from heavy metal damage. Upon exposure to heavy metals, cellular expression of metallothioneins is markedly up-regulated as part of the stress response. Metallothioneins also bind zinc as part of the system for maintaining zinc homeostasis and are thus sensitive to certain hormones. Karin studied the regulation of metallothionein by heavy metals and also glucocorticoid hormones (2). "When I started, molecular cloning was not a household word," he says. He therefore studied the regulation of metallothionein synthesis in HeLa cells by using radioactive labeling of newly synthesized metallothionein protein. Later on, a postdoctoral fellow, Bob Anderson, showed Karin a new technique for *in vitro* translation, providing "a very cumbersome way to measure mRNA," says Karin. But the technique produced results. Karin showed that metallothionein mRNA production is induced by dexamethasone and zinc (3).

Metallothionein on the Move

After receiving his Ph.D. in molecular biology in 1979, Karin moved on to postdoctoral studies. For his first postdoctoral position, Karin recalls that Herschman encouraged him to work with Beatrice Mintz at the Fox Chase Institute for Cancer Research (Philadelphia, PA). Mintz was studying embryonal carcinomas and developing methods for generating transgenic mice. With Mintz, Karin generated a mouse model with a deficiency in the transferrin receptor (4). "But in order to do this, we needed to know more about the receptor and how it functions," he says. Karin felt the project focused more on the biochemistry of iron uptake rather than on the genetics, and he was eager to get back to studying genetic regulation.

In 1980, Karin moved on to another postdoctoral position at the University of California, San Francisco (UCSF), with John Baxter, whom Karin calls a pioneer in the fields of nuclear receptors and molecular endocrinology. At the time, UCSF was what Karin describes as a "hotbed of molecular cloning." Recombinant DNA technology had just become available, and Karin began to look at the regulation of growth hormone gene expression by glucocorticoid hormones. Baxter had pituitary cell lines that expressed growth hormone, but Karin tried to transfect the growth hormone gene into fibroblasts. Transfection was a new technique at the time, and the experiments were unsuccessful in duplicating growth hormone gene regulation. Karin went back to studying the same questions of hormonal regulation of transcription but with the more tractable metallothionein genes. He cloned the human metal-

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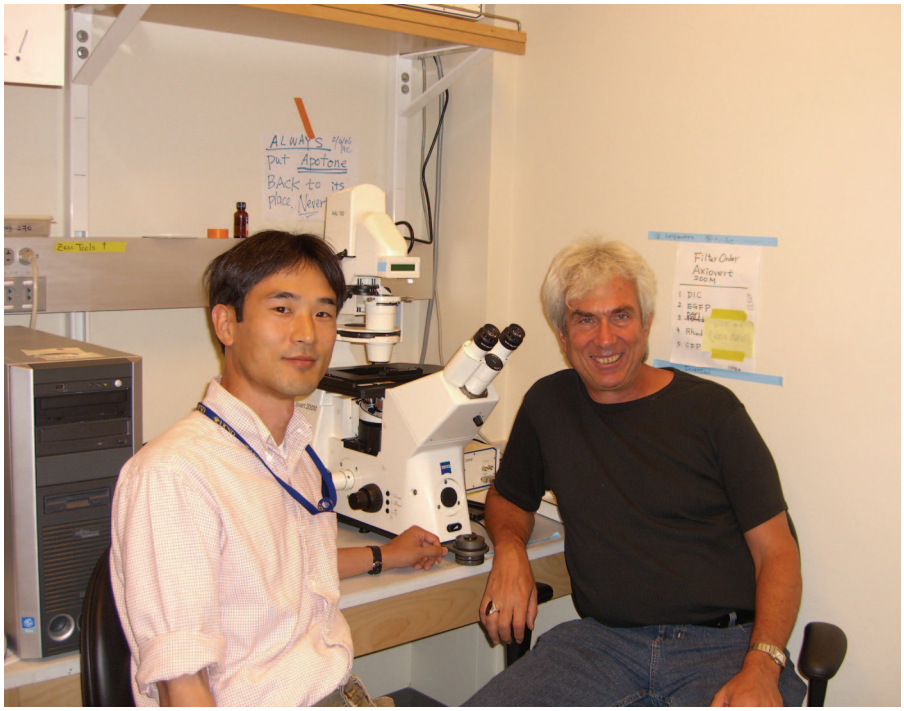
This is a Profile of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 10544 in issue 28 of volume 103.

lothionein genes and characterized them to look at how the genes were regulated (5). The metallothionein project followed Karin in his next move, as did the quest for signals that regulate gene activity.

In 1982, Karin joined the faculty of the University of Southern California (USC; Los Angeles, CA) as an Assistant Professor of Microbiology. "That was the first place to offer me a fully independent position. I took the first offer," he says. Karin found himself with a team of highly dedicated workers early on. "I was very lucky to have an outstanding group of postdocs and graduate students when I had just started. It's unusual for a freshly minted assistant professor," he says. Karin notes that this team made a couple of major contributions to the field, one being the first dissection of a promoter for an inducible cellular gene. Karin says that most work on mammalian promoters focused on viral systems. In a promoter, cis-acting elements are the sites on DNA to which trans-acting proteins bind to regulate promoter activity and gene expression. The trans-acting factors receive signals from inside and outside the cell that regulate their activity and thereby modulate gene expression. Karin began to look at the promoter region of metallothionein IIA. He mutated the promoter region in various places to determine which regions governed various activities and responses, from basal activity to heavy metal ion and hormonal induction (6). "The metallothionein promoter is packed with cis elements that allow it to respond to many signals. It all seems very obvious now, but that was not the case then," he says (7).

The second major contribution made by Karin and his team came about with the help of a new postdoctoral fellow, who happened to be a high school classmate of Karin's. Mordechai Bodner had stayed in Israel for his Ph.D. work and then joined Karin to look at growth hormone gene expression. Together, Karin and Bodner developed the first cell-type-specific *in vitro* gene expression system. The work showed that expression of cell-type-specific genes could be studied *in vitro*, not just in cells (8).

In 1986, Karin accepted a faculty position at UCSD and moved his laboratory there quickly. "We packed the lab in a week," he says. All of the postdoctoral fellows helped pack and drive the boxes to UCSD in what Karin recalls as a "low-budget" move. At UCSD, work continued on the first cell-type-specific transcription factor, as well as on the metallothionein promoter. With collaborator Robert Tjian at the University of California, Berkeley, Karin mapped the transcription factor binding sites in the metallothionein promoter (9).



Karin, with Inaugural Article lead author Toshi Sakurai, examining liver sections for presence of tumors.

One of Karin's postdoctoral fellows, Peter Angel, began studying how genes were regulated by phorbol ester tumor promoters. Angel, Karin, and colleagues found that activator protein 1 (AP-1) mediated this response (10). AP-1 is a trans-acting factor that recognized one of the cis elements from the metallothionein promoter, as well as the collagenase gene, which Angel studied as a student. Karin recalls that "the full meaning of these results became clear a year or so later when we had purified AP-1 and looked at its components. AP-1 is a complex of different transcription factors, Jun and Fos," he says (11). Fos was already known as a human oncogene, and Jun had just been described as an oncogene by Peter Vogt, Karin's former department chair at USC (12). "Having found that, it was clear that some protooncogenes code for proteins that act in the nucleus. This was just the beginning of understanding that some oncogenes code for transcription factors that somehow stimulate cell proliferation," says Karin. The work also raised the possibility that AP-1 was a major target for growth-promoting signals, a topic that, almost 20 years later, still occupies Karin's laboratory.

NF-κB: Following Pathways Upstream

Karin was happy to have landed at UCSD. "San Diego is one of the best places to study signal transduction. Signal transduction was not a common term in the early 1980s, especially not protein phosphorylation," he says. At the time, Karin wanted to know what regulated AP-1 in response to growth factors. He collaborated with Tony Hunter at The Salk Institute (La Jolla, CA), and together they studied which protein kinases regulated AP-1 activity. The work "allowed us to identify phosphorylation events that regulate the activity of AP-1," explains Karin (13). From this point, Karin's group went on to identify Jun kinases (JNKs) and the entire pathway that regulates their activity (14, 15).

Frank Mercurio, one of Karin's first graduate students at UCSD, helped Karin work on another cis element, recognized by a trans-acting factor called AP-3 at the time and now known as NF-κB. In the early 1990s, Mercurio was Karin's only student working on this project, limiting the amount of research that could be done. Rather than trying to characterize all of the members of the NF-κB family, Karin and Mercurio instead focused on its regulation. Two postdoctoral fellows, Joe DiDonato and David Rothwarf, continued the work, which culminated with identification of the IKK complex (16). "That very quickly became the major topic of studies in my lab. It is something that everyone was looking for. It really allowed us to understand how NF-κB is regulated," says Karin.

The function of NF-κB as a modulator of the immune response, although still generalized, is more specific than AP-1, explains Karin, so his laboratory's focus shifted. They worked on generating knockout mice deficient in subunits of IKK and found that "IKKα has a very critical role in the development of the skin," says Karin (17). Also, IKKβ was found to be important for the inhibition of apoptosis, or programmed cell death (18).

The latter finding would steer Karin's laboratory in another new direction, to chase another pathway. "This got us thinking about inflammation and cancer," he says. NF-κB is important for inflammation and can block apoptosis, and, given its role, Karin speculated that NF-κB may link inflammation and cancer. In a review article, he provided a lot of circumstantial evidence from the literature that NF-κB could indeed provide such a function (19). "This led us to set up experimental tools and systems to study that more rigorously," he says.

A mouse model of colorectal cancer provided the first system. Colitis, or inflammation of the colon, had been known to increase the risk of colorectal cancer. Using a mouse model with a conditional knockout of the IKKβ subunit, Karin and his team found that NF-κB had two important functions in cancer, both dependent on cell type. "When NF-κB is activated in intestinal epithelial cells, its most important function is to suppress apoptosis," he says. "However, it also promotes tumor development when activated in macrophages because it stimulates production of growth factors, or mitogens (20)."

Having found these results, Karin and his group decided to look at another model. They used the same strategy as with colitis-associated cancer but with inactivated IKKβ in hepatocytes to study liver cancer, or hepatocellular carcinoma. "The big surprise was that we ended up with more cancer with IKKβ and NF-κB inactivation," says Karin.

"We have a lot more to do with liver cancer."

A few years ago, a former postdoctoral fellow, Anning Lin, now a professor at the University of Chicago (Chicago, IL), found that when NF-κB activation was prevented, JNK1 sustained prolonged activation. Normally, JNK1 is activated transiently and shut off fairly quickly. According to Karin, "JNK1 activation is important not only for killing hepatocytes but also for their proliferation. As more hepatocytes die in response to carcinogen exposure, more of the remaining cells are triggered to proliferate to compensate for the lost cells. This compensatory proliferation is probably responsible for liver cancers not only in mice but also in humans," he says.

In his PNAS Inaugural Article (1), Karin and his team investigated the JNK1 pathway further and showed that blocking JNK1 could reverse liver carcinogenesis. Karin's team used mice homozygous for a JNK1 deficiency on both a wild-type and an IKKβ-hepatocyte-deficient background. Upon addition of a carcinogen (diethylnitrosamine), both mouse strains developed liver cancer. Those mice that lacked IKKβ in hepatocytes developed more tumors, but removing JNK1 from the system reversed this increase and brought the cancer load back to what they had seen in wild-type mice. Also, although the removal of IKKβ enhanced compensatory proliferation, the removal of JNK1 abrogated the proliferative response.

Despite these advanced findings, Karin says that he is not ready to stop studying hepatocytes or JNK1 any time soon. "We have a lot more to do with liver cancer. We want to confirm that the same thing is happening in humans," he says. But the proposed work will not be easy, according to Karin. "Logistically, it's not that simple," he says, explaining that procuring samples and collaborators takes time. "We can do lots of things in mice, but we want to know that what we're showing in mice is relevant to humans," he says. So Karin continues to trace the roots of genetic regulation, with each new project leading into the next, winding his way through the genome, following one thread of regulation after another.

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1. Sakurai, T., Maeda, S., Chang, L. & Karin, M. (2006) *Proc. Natl. Acad. Sci. USA* **103,** 10544–10551.
2. Karin, M. & Herschman, H. R. (1979) *Science* **204,**176–177.
3. Karin, M., Andersen, R. D., Slater, E., Smith, K. & Herschman, H. R. (1980) *Nature* **286,** 295–297.
4. Karin, M. & Mintz, B. (1981) *J. Biol. Chem.* **256,** 3245–3252.
5. Karin, M. & Richards, R. I. (1982) *Nucleic Acids Res.* **10,** 3165–3173.
6. Karin, M., Haslinger, A., Holtgreve, H., Cathala, G., Slater, E. & Baxter, J. D. (1984) *Cell* **36,** 371–379.
7. Karin, M., Haslinger, A., Holtgreve, H., Richards, R. I., Krauter, P., Westphal, H. M. & Beato, M. (1984) *Nature* **308,** 513–519.
8. Bodner, M. & Karin, M. (1987) *Cell* **50,** 267–275.
9. Lee, W., Haslinger, A., Karin, M. & Tjian, R. (1987) *Nature* **325,** 368–372.
10. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) *Cell* **49,** 729–739.
11. Chiu, R., Boyle, W. J., Meek, J., Smeal, T., Hunter, T. & Karin, M. (1988) *Cell* **54,** 541–552.
12. Vogt, P. K., Bos, T. J. & Doolittle, R. F. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3316–3319.
13. Lin, A., Frost, J., Deng, T., Al-Alawi, N., Smeal, T., Kikkawa, U., Hunter, T., Brenner, D. & Karin, M. (1992) *Cell* **70,** 777–789.
14. Hibi, M., Lin, A., Smeal, T., Minden, A. & Karin, M. (1993) *Genes Dev.* **7,** 2135–2148.
15. Lin, A., Minden, A., Martinetto, H., Claret, F.-X., Lange-Carter, C., Mercurio, F., Johnson, G. L. & Karin, M. (1995) *Science* **268,** 286–290.
16. DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E. & Karin, M. (1997) *Nature* **388,** 548–554.
17. Hu, Y., Baud, V., Delhase, M., Zhang, P., Deerinck, T., Ellisman, M., Johnson, R. & Karin, M. (1999) *Science* **284,** 316–320.
18. Li, Z.-W., Chu, W., Hu, Y., Delhase, M., Deerinck, T., Ellisman, M., Johnson, R. & Karin, M. (1999) *J. Exp. Med*. **189,** 1839–1845.
19. Karin, M., Cao Y., Greten, F. & Li, Z.-W. (2002) *Nat. Rev. Cancer* **2,** 301–310.
20. Greten, F. R., Eckmann, L., Greten, T. F., Park, J. M., Li, Z.-W., Egan, L. J., Kagnoff, M. F. & Karin, M. (2004) *Cell* **118,** 285–296.